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Postpartum Estrous Cyclicity and Fertility in Cross

Aashirbad Pokharel[1], Gokarna Gautam[1@]
[1] Department of Theriogenology, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Bharatpur-15, Chitwan, Nepal: AP: aashirbad.pokharel@gmail.com
[@1]Department of Theriogenology, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Bharatpur-15, Chitwan, Nepal: @ggautam@afu.edu.np,
https://orcid.org/0000-0002-2020-0902;

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ABSTRACT

This study determined the effect of various factors on the resumption of postpartum (pp) estrous cyclicity within 60 days (d) pp and 90 d pp and pregnancy within 90 d pp in cross-bred dairy cows. Blood was sampled (n=46) once during 50-60 d pp to determine serum glucose, total protein, albumin, urea, blood urea nitrogen and creatinine concentration. Body condition score (BCS) was determined twice- during 50-60 and 80-90 d pp. A total of 16 (34.8%) cows resumed estrous cyclicity within 60 days, and 30 (65.2%) cows resumed estrous cyclicity cumulatively within 90 days postpartum. More cows with BCS>2.50 as compared to cows with BCS≤2.50 during 50-60 d pp resumed estrus within 60 d pp (P<0.05). More cows without periparturient problems-initiated estrus within 60 d pp as compared to cows with periparturient problems (P<0.05). BCS change (from 50-60 to 80-90 d pp) and periparturient problems significantly influenced estrus resumption within 90 d pp (P<0.05). However, breed, parity and milk yield had no influence on the resumption of estrus cyclicity either within 60 d pp or 90 d pp. Among various blood nutritional metabolic parameters, only the serum albumin influenced estrus resumption within 60 d pp (P<0.05). Pregnancy within 90 d pp was significantly influenced by BCS, BCS change and periparturient problems (P<0.05). In conclusion, the resumption of estrous cyclicity postpartum was affected by the periparturient problems, BCS, BCS change and serum albumin level, whereas the pregnancy within 90 d pp was affected by the periparturient problems, BCS and BCS change.

Keywords: Albumin, Blood nutritional metabolic parameters, Body condition score (BCS), Periparturient problems, Postpartum estrous cyclicity

सारांश

यस अध्ययनमा क्रस-ब्रिड डेरी गाईहरूमा प्रसूति पछि (पोष्टपार्टम, pp) ऋतु चक्रपुनः सुरु हुने समय र ९० दिन भित्र गर्भधारणमा विभिन्न कारकहरूको प्रभाव हेरिएको थियो। अध्ययन अनुरुप, ५०-६० दिन pp मा रगतको नमुनाहरु (n=४६) संकलन गरी सिरम ग्लुकोज, कुल प्रोटिन, एल्बुमिन, यूरिया, ब्लड यूरिया नाइट्रोजन र क्रिएटिनिनको स्तर मापन गरिएको थियो। साथै, शरीरको अवस्था (Body Condition Score, BCS) ५०-६० र ८०-९० दिन pp मा जाँचिएको थियो। अध्ययनमा जम्मा १६ (३४.८%) गाईहरूले ६० दिनभित्र ऋतु चक्र पुनः सुरु गरेका थिए भने, ९० दिनभित्र कुल ३० (६५.२%) गाईहरूले ऋतु चक्र पुनः सुरु गरेका थिए। ५०-६० दिन pp मा BCS>२.५० भएका गाईहरूले BCS≤२.५० भएका गाईहरूको तुलनामा ६० दिन भित्र बढीले ऋतु चक्र सुरु गरे। साथै, प्रसूति सम्बन्धी समस्या नभएका गाईहरूले समस्या भएका गाईहरूको तुलनामा छिटो ऋतु चक्र सुरु गरे। BCS परिवर्तन (५०-६० देखि ८०-९० दिन pp) र प्रसूति सम्बन्धी समस्याले ९० दिन भित्र ऋतु चक्र सुरु हुनमा असर पुर्याएको पाईयो। तर, जात, parity र दूध उत्पादनले ऋतु चक्र पुनः सुरु हुनमा असर गरेको पाइएन। रगतको पोषण सम्बन्धी प्यारामिटरमध्ये सिरम एल्बुमिन मात्र ६० दिन भित्र ऋतुचक्र सुरु हुनमा प्रभावकारी थियो। ९० दिन pp भित्र गर्भधारणमा BCS, BCS परिवर्तन र प्रसूति समस्याको असर महत्वपूर्ण थिए। निष्कर्षमा, प्रसूति पछि ऋतु चक्र पुनः सुरु हुनुमा शरीरको अवस्था,

BCS परिवर्तन, एल्बुमिन स्तर र प्रसूति समस्याले प्रभाव पार्छ भने ९० दिन भित्र गर्भधारण हुनेमा मुख्य असर BCS, BCS परिवर्तन र प्रसूति समस्याले पुऱ्याउँछ।

INTRODUCTION

Though the improved nutrition, genetics and management have accomplished high milk production (>10,000 kg/cow) and long lactation days (305 days), its consequences have also led to decreased reproductive efficiency in cows (Cole et al 2023). In the dairy industry, high fertility and successful breeding are the key factors (Peter et al 2009). However, declining fertility over the last few years has been the most alarming problem in dairy cows (Walsh et al 2011). Delay in postpartum resumption of ovarian activity and ovulation is one of the most important reproductive problems leading to low conception rate, embryonic loss, and increased calving interval (Shrestha et al 2004). A few days postpartum anestrus is considered normal physiology, whereas failure to return to estrus after 60 days postpartum is regarded as pathological anestrus (Islam et al 2014). To obtain an optimal calving interval which is about one year, the postpartum cow should recommence its ovarian activity, come into estrus, be mated, and conceive within 85 days postpartum (Inchaisri et al 2010). There have been approaches to treat postpartum anestrus in crossbred dairy cows; however, the problem remains a major concern (Gautam et al 2019).

There are several factors affecting the resumption of the postpartum estrous cycle in dairy cows. The presence of periparturient diseases such as abortion, ketosis, mastitis, retained placenta, and metritis had a delayed resumption of estrus cyclicity with an increased risk of pregnancy loss (Ribeiro et al 2013). A delayed postpartum estrus resumption has been demonstrated in the cows losing ≥ 1 unit body condition score (BCS) after calving (Shrestha et al 2005). Findings of the previous studies on the effect of parity on resumption of estrous cyclicity postpartum in cattle are inconsistent; some authors observed that postpartum ovarian activity was not influenced by parity (Bulman and Lamming 1978; Sonam et al 2020), while others reported more primiparous anestrous cows than pluriparous resumed estrous cyclicity in the first 60–70 days postpartum (Moreira et al 2001; Rhodes et al 2003). Moreover, there were inconsistent findings regarding the association of blood metabolic profiles with the resumption of estrous cyclicity postpartum and subsequent pregnancy in dairy cows (Damptey et al 2014; Guzel and Tanriverdi 2014; Obese et al 2015). Furthermore, the effect of breed and milk yield on resumption of estrous cyclicity postpartum and subsequent pregnancy in not clearly established. Therefore, the objective of this study was to determine the effect of various factors on the resumption of postpartum estrous cyclicity and subsequent pregnancy in dairy cattle.

MATERIALS AND METHODS

Study site and animals

The study was conducted in Shree Laligurans Dairy Farm & Research Center, located at Khairahani-12, Chitwan, Nepal (27.571°N, 84.571°E). The area has a tropical monsoon climate with three distinct seasons, with a peak summer temperature of 42°C and a winter minimum temperature of 3°C. There were over 600 cows including parous, heifers, and calves. The parous cows were kept under different roofed structures in loose housing system according to the stage of lactation. The feed, feeding system, and management practice were maintained the same for all cows in the experiment. Of the total number of cows in the farm, the cows in 50 to 60 days postpartum (d pp) (n=46) were selected for this study. Mean (±SE) parity of the cows in the experiment was 2.51±1.05 (Range: 1 to 4) and the mean (±SE) daily milk yield was 8.44±3.23 kg.

Data collection

Data regarding parity, periparturient problems (abortion, ketosis, mastitis, retained placenta, metritis), milk yield, and date of estrus were collected from farm records. The breed of the cow was noted by observing the phenotypic characteristics of the cow.

Body condition score

During 50-60 d pp, the body condition score (BCS) of each cow was determined on 1-5 scale with 0.25 increments (Edmonson et al 1989; Ferguson 1994). The determination of BCS was repeated at 80-90 d pp (i.e., one month after the first examination). Then unit and percentile BCS changes and body weight changes were calculated.

Blood collection for determination of blood nutritional parameters

Simultaneous to body condition score, during 50-60 d pp, the blood sample from each cow was collected from jugular vein into serum tubes. The samples were transported to the Laboratory of Theriogenology, Agriculture and Forestry University (AFU) keeping them in a box with ice packs. At the laboratory, serum was separated by centrifuging the blood samples at 1,000 x g for 15 minutes. Blood nutritional parameters- glucose, total protein, urea, albumin, blood urea nitrogen (BUN), blood urea, SGOT, SGPT and creatinine were determined using automatic photoelectric colorimetry (Automatic PKL PPC 125, Changchun Zyf Science and Technology Co., Ltd., China).

Faecal examination

The fecal samples were collected once during 50-60 d pp directly from the rectum by palpation, packed in a zip-lock bag and the samples were transported to the laboratory of Theriogenology, AFU in a cool box. The sedimentation method was used to observe the eggs of gastrointestinal parasites, if any. After observation, data were recorded as the absence or presence of gastrointestinal parasites.

Recording of estrus within 60 and 90 days postpartum

As the cows were kept under a free housing system, the estrus detection was done by observing the standing to be mounted and vaginal mucus discharge. The detection was done thrice a day and was a routine procedure of the farm. The date of estrus of each cow was noted from farm records, and the cows were categorized into following groups as:

- Those coming into estrus within 60 d pp and those not coming into estrus within 60 d pp
- Those coming into estrus within 90 d pp and those not coming into estrus within 90 d pp

Pregnancy diagnosis

Cows in the study were followed at least up to 130 days postpartum to determine whether the cows were pregnant within 90 days postpartum. Pregnancy was diagnosed using the transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China).

Statistical analysis

Data were analyzed using SPSS (Version 25.0). The effect of various factors on the resumption of estrus cyclicity as well as on pregnancy rate within 60 and 90 days postpartum was analyzed using a chi-square test. Various blood metabolic parameters between two groups of cows were compared using a t-test. P value ≤ 0.05 was considered as significant whereas $0.05<P\leq 0.1$ was considered to have tendency effect.

RESULTS

Out of 46 cows, 16 (34.8%) cows resumed estrous cyclicity within 60 days postpartum. Upon fecal examination, no gastrointestinal parasites were found, which may be due to timely and effective deworming practices at the farm. Factors affecting the resumption of estrus cyclicity within 60 d pp have been shown in **Table** 1. Among the various factors considered in the analysis, only the BCS and periparturient diseases had significant effect on resumption of estrous cyclicity within 60 d pp. Among the cows of interest, 20 had periparturient diseases and 26 cows had no problems. The periparturient problems included mastitis, abortion, stillbirth, cervicitis and post-FMD complications. The proportion of cows that resumed estrous cyclicity within 60 d pp was 10.0% (2/20) and 53.9% (14/26), respectively among the cows with and without periparturient diseases (P = 0.03). Likewise, only 9.1% (1/11) cows with poor BCS (≤2.50) during 50-60 d pp as compared to 42.9% (15/35) cows with good BCS (>2.50) during 50-60 d pp resumed estrous cyclicity within 60 d pp.

Table 1. Factors affecting resumption of estrus cycle by 60 d pp in dairy cows

Variable		Number	Resumption of estrus cycle ≤ 60 d pp, n (%)	P-value
Breed	Jersey cross	11	5 (45.5)	0.39
	Holstein Friesian cross	35	11 (31.4)	
Parity	Primiparous	8	1 (12.5)	0.15
	Pluriparous	38	15 (39.5)	
Periparturient diseases	Yes	20	2 (10.0)	**0.03**
	No	26	14 (53.9)	
Milk yield	<average (<8.4kg/d)	20	6 (30.0)	0.77
	≥average (≥8.4kg/d)	26	10 (38.4)	
BCS at 50-60 d pp	>2.50	35	15 (42.9)	**0.04**
	≤2.50	11	1 (9.1)	

Factors affecting the resumption of estrus cyclicity by 90 days postpartum have been shown in Table 2. Overall, 30 of 46 cows (65.2%) resumed estrous cyclicity cumulatively by 90 days postpartum, whereas 16 cows (34.8%) did not resume estrus within this period. Among the various factors, the periparturient diseases and BCS change (from 50-60 to 80-90 d pp) had significant effect on resumption of estrous cyclicity within 90 d pp. A total of 92.3% (24/26) of cows with no periparturient problems versus 30.0% (6/20) of cows with periparturient problems resumed the estrous cyclicity within 90 d pp ($P < 0.05$). There were 14 cows with increased BCS from 60 to 90 days of postpartum. The number of cows without any change or decreased BCS during that period was 16. The proportion of cows that resumed estrous cyclicity within 90 days (85.7% vs 12.5%; P=0.021) was higher among the cows that had an increase in BCS from 60 to 90 d pp than in cows that had no change or decrease in BCS from 60 to 90 d pp. The mean (±SE) BCS change for the cows coming and not coming to estrus within 90 days was 0.25±0.05 and 0.09±0.1, respectively (P=0.08).

Table 2. Factors affecting resumption of estrus cycle by 90 days pp in dairy cows

Variable		Number	Resumption of estrus cycle ≤ 90 d pp, n (%)	*P-value*
Breed	Jersey cross	11	9 (81.8)	0.60
	Holstein Friesian cross	35	21 (60.0)	
Parity	Primiparous	8	4 (50.0)	0.75
	Pluriparous	38	26 (68.4)	
Periparturient diseases	Yes	20	6 (30.0)	**0.04**
	No	26	24 (92.3)	
Milk yield	<average (<8.4kg/d)	20	13 (65.0)	1.00
	≥average (≥8.4kg/d)	26	17 (65.4)	
BCS at 50-60 d pp	>2.50	35	27 (77.1)	0.15
	≤2.50	11	3 (27.3)	
BCS change from 60-90 d pp[a]	No change/Decreased	16	2 (12.5)	**0.02**
	Increased	14	12 (85.7)	

[a] The number of cows for BCS change is 30 instead of 46. This is because 16 cows that resumed estrus cyclicity within 60 d pp were excluded for this parameter.

The mean (±SE) of the different blood metabolic parameters (during 50-60 d pp) of the cows with and without resumption of estrous cyclicity within 90 days postpartum has been shown in **Table** 3. Among the various blood metabolic parameters, albumin was significantly higher (P = 0.04) in cows that resumed estrous cyclicity within 90 d pp than cows that did not resume estrus cyclicity within 90 d pp. Whereas, other parameters were not significantly different between cows that resumed and did not resume estrus cyclicity within 90 d pp.

Table 3. Blood serum parameters (Mean±SE) during 50-60 d pp in cows that came and did not come to estrus ≤60 d pp

Parameters (Mean±SE)	Cows in estrus ≤60 d pp (n=16)	Cows not in estrus ≤60 d pp (n=30)	*P*-value
Glucose (mg/dl)	51.5±3.6	47.1±3.9	0.22
Total protein (g/dl)	7.2±0.2	7.1±0.2	0.49
Albumin (g/dl)	3.2±0.0	2.9±0.1	**0.04**
Creatinine (mg/dl)	1.0±0.1	1.1±0.1	0.26
BUN (mg/dl)	12.2±0.4	12.3±0.4	0.41
Urea (mg/dl)	27.3±0.8	26.4±0.8	0.23
SGOT (U/L)	83.5±8.1	82.1±7.3	0.45
SGPT (U/L)	30.6±4.5	26±2.	0.17

A total of 28.3% (13 out of 46) of cows became pregnant within 60 d pp. Additional 12 cows conceived between 60 and 90 d pp. Thus, the overall pregnancy rate reached 54.3% (25 out of 46) within 90 d pp. In accordance with estrus cycle resumption, 81.3% (13 out of 16) cows that resumed estrus cyclicity within 60 d pp became pregnant. Similarly, 83.3% (25 out of 30) cows that resumed estrus cyclicity within 90 d pp became pregnant.

Effect of various factors on pregnancy by 90 d pp has been shown in **Table** 4. Periparturient diseases, BCS during 50-60 d pp and BCS change from 60 to 90 d pp significantly affected the pregnancy rate. Cows with periparturient diseases had lower pregnancy within 90 d pp as compared to that without periparturient diseases (20.0% vs 80.8%; P=0.03). Likewise, pregnancy rate was significantly higher (P=0.006) in cows with good BCS (>2.50) during 50-60 d pp as compared to cows with poor BCS (≤2.50) during 50-60 d pp. Similarly, the proportion of cows that were pregnant (86.4% vs 25.0%; P=0.02) was higher among the cows that had an increase in BCS from 60 to 90 d pp than in cows that had no change or decrease in BCS from 60 to 90 d pp.

Table 4. Effect of various factors on pregnancy by 90 d pp in cross-bred dairy cows

Variable		Number	Pregnancy, n (%)	*P*-value
Breed	Jersey cross	11	8 (72.7)	0.58
	Holstein Friesian cross	35	17 (48.6)	
Parity	Primiparous	8	3 (37.5)	0.44
	Pluriparous	38	22 (57.9)	
Periparturient diseases	Yes	20	4 (20.0)	**0.03**
	No	26	21 (80.8)	
Milk yield	<average (<8.4kg/d)	20	10 (50.0)	0.81
	≥average (≥8.4kg/d)	26	15 (57.7)	
BCS at 50-60 d pp	>2.50	35	25 (71.4)	**0.01**
	≤2.50	11	0 (0.0)	
BCS change from 60-90 d pp	No change/Decreased	24	6 (25.0)	**0.02**
	Increased	22	19 (86.4)	

DISCUSSION

The ability to resume estrous cycles early postpartum and maintain pregnancy influences the reproductive performance of dairy cows (Santos et al 2004). Although delayed postpartum ovulation is associated with a more pronounced negative energy balance, other factors might also be involved (Butler 2003). Therefore, this study was conducted to investigate the effect of various factors on the estrus cyclicity resumption and subsequent pregnancy rate.

There was no significant effect of breed on estrus resumption both within 60 d pp and 90 d pp in this study. This may be because both the breeds used in this study were exotic with similar production potential. Similar results were observed in the previous studies (Pereira et al 1995; Saha et al 2016). Reports regarding the effect of parity on uterine involution and estrous resumption in cattle have been inconsistent. In the present study, there was no effect of parity on the resumption of estrous cyclicity either within 60 d pp or 90 d pp. This result was in agreement with the findings of the previous studies those observed that postpartum ovarian activity was not influenced by parity (Bulman and Lamming 1978; Sonam et al 2020). However, others have reported more primiparous anestrous cows than multiparous in the first 60–70 days postpartum (Moreira et al 2001; Rhodes et al 2003). Primiparous cows have greater concentrations of blood non-esterified fatty acids than multiparous cows early postpartum (Meikle et al 2004) and have more early postpartum uterine problems (Goshen and Shpigel 2006), resulting delay in the resumption of postpartum ovulation.

In the present study, cows without periparturient diseases resumed estrous cyclicity earlier than the cows having diseases. The cows with disease had higher risk of delayed estrus resumption within both 60 days and 90 days pp compared to the cows without diseases. Postpartum infections associated with abnormal puerperium suppress the hypothalamic release of gonadotropin-releasing hormone, consequently delaying folliculogenesis (Bosu et al 1988). Delayed ovarian resumption and reduced conception rate in cows with postpartum endometritis have also been reported (Lourens 1995). In this study, there was no statistically significant difference in estrus resumption between cows with high and low milk production either within 60 d pp or within 90 d pp. Increased milk yield could delay the onset of estrous cycles if associated with impaired nutrient balance, mainly related to dry matter intake and energy intake (Liefers et al 2003). Cows with less production could be those having periparturient diseases which in addition to reduction in lactation also delays the postpartum ovulation (Goshen and Shpigel 2006).

Negative energy balance has been negatively associated with the ability of lactating dairy cows to conceive and maintain a pregnancy to term. In this study, the cows with BCS >2.50 had a significantly higher estrus resumption rate as compared to the cows with BCS≤2.50. Also, as the BCS increased, the proportion of estrual cows also increased. Further, a loss in BCS or no increment in BCS markedly reduced the proportion of cows that had initiated estrous cycles. As body reserves are utilized during periods of energy shortage, BCS serves as a practical indicator of the energy status of dairy cows and is closely associated with their ability to resume estrous cyclicity (Shrestha et al 2005).

In this study, no significant difference was obtained in the serum glucose level between the cows with and without the resumption of the estrous cycle. This is supported by the research done by Obese et al (2015), who also stated no relation existed between serum glucose and cyclicity resumption in cows. A steady plasma glucose both during calving and postpartum was found (Abdulkareem 2013). No significant difference in the serum total protein between the groups was obtained in this study. This is similar to the result reported by Damptey et al (2014). However, a significantly higher total protein level in non-cyclic than cyclic cows was found in the study conducted (Guzel and Tanriverdi 2014). No significant difference in blood urea level was obtained between the groups. This contrasts with the study by Paiano et al (2018) who reported it to be lower from 2 to 6 weeks postpartum in non-cyclic cows arising due to reduced dietary protein intake and/or reduced hepatic urea genesis. This higher level of urea level was observed in cows with parasitic infection (Obese et al 2015). No parasitic infestation upon fecal examination was observed in the cows in this study, which maybe the reason for similar blood urea level between the cows that resumed and did not resume estrus cyclicity. The cows that resumed the estrus cycle within 60 days postpartum had serum albumin levels significantly higher than that of cows not resuming the estrus cycle. A similar result was obtained from the study done by Obese et al (2015). In cattle, the blood albumin level is associated with the good nutritional status and good BCS. Therefore, the higher level of albumin in cows with early estrus resumption might be because of the better BCS as compared to the cows with late estrus resumption (Coppo 2004).

Though some authors have found greater conception rates for primiparous than multiparous cows (Tenhagen et al 2004), no significant difference was obtained in this research. A significant influence ($p<0.05$) of periparturient diseases on pregnancy rate was established in this study such that the pregnancy rate of the cows without PP diseases higher than that of cows having the diseases. Several similar reports have been documented that state the low conception rate with high embryonic mortality in cows with periparturient problems (Lavon et al 2011; Ouweltjes et al 1996; Vieira-Neto et al 2014). Similarly, in the current study, a similar conception rate was observed between the cows producing milk less and more than or equal to average. This result was in align with the result obtained by (Chebel et al 2004) who stated no association of milk yield with conception or embryonic survival. Both BCS and the change in BCS had a significant impact on pregnancy or conception rate in this study. Development and maintenance of pregnancy is influenced by the BCS (López-Gatius et al 2002). Early postpartum BCS changes were important indicators of the risk of conception at first AI in dairy cows (Domecq et al 1997). Cows with excessive BCS loss in early lactation had prolonged intervals to the first AI and were less likely to conceive (Domecq et al 1997).

CONCLUSION

BCS, periparturient diseases, and BCS change were associated with the resumption of the estrous cycle within 90 days of postpartum. Only the serum albumin among the blood parameters significantly influenced the postpartum estrous resumption. Similarly, BCS, BCS change, and periparturient diseases significantly affected the conception rate up to 90 days postpartum. Timely evaluation of the BCS postpartum helps to identify the cows with poor nutritional status and improve nutritional management.

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