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Association of certain serum metabolic markers and macro-minerals on resumption of ovarian activity in postpartum crossbred dairy cows

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Abstract

The study was conducted to identify the factors influencing resumption of cyclicity in postpartum crossbred dairy cows. Crossbred dairy cows (n=40) were selected for study and the basic details on age, parity, body condition score (BCS), peripartum complications and milk production were recorded. During the study period of 45 to 90 days postpartum, clinico-gynaecological examination of animals were conducted at 15 days interval to record ovarian and uterine changes. Serum progesterone levels were estimated at 15 days interval starting from day 45 postpartum and the animals with progesterone concentration of > 1 ng/mL were considered as resumed ovarian activity. Serum macro-minerals and metabolites including Ca, P, Mg, total protein (TP) and cholesterol levels were estimated during various postpartum periods and its influence on resumption of cyclicity in crossbred dairy cows were analysed. The BCS and serum Ca concentration of cows which resumed ovarian cyclicity were significantly ($p<0.01$) higher than that of cows which did not resume cyclicity. Serum TP ($p<0.01$) and cholesterol ($p<0.05$) were significantly higher in resumed group of cows on day 75 postpartum compared to the not resumed group. Group of cows which resumed postpartum ovarian cyclicity showed significantly ($p<0.01$) lower milk production than that of cows which did not resume cyclicity. No significant association of serum P and Mg levels with resumption of ovarian activity was observed.

Keywords: *Postpartum dairy cows, resumption of ovarian activity, macro minerals*

The transition period after calving is a critical phase in dairy cows, where the cows shift from pregnancy to lactation. Significant physiological changes occur during this period, which are influenced by various factors such as breed, age, milk yield, nutrition, body condition and season (Stevenson *et al*., 1997). During the transition period there is a complex interplay of reproductive and metabolic processes which could influence the overall health and productivity of the animal. Negative energy balance (NEB) during the postpartum period will affect the reproductive performance of dairy cows which is marked by a delayed resumption of ovarian activity (ROA) (Stevenson *et al.*, 1997). The present study was designed to explore the association between postpartum resumption of ovarian activity and macro-mineral status of crossbred cows and to assess the importance of blood metabolic markers as risk factors for postpartum anoestrus.

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Major factors influencing resumption of ovarian activity in dairy cows studied in this research were age, parity, body condition score (BCS), milk production and concentration of many macro-minerals and metabolites in the body. During the transition period, dairy cows often faced metabolic disorders, particularly when their intake of glucose, protein or calcium was insufficient, negative energy balance, prevalent from late pre-calving period through the first 4 to 6 weeks post-partum, led to increased lipid mobilisation and subsequent disorders like ketosis and fatty liver (Van Saun, 1997). In high-producing cows severe negative energy balance impaired glucose homeostasis, leading to ketone body production as an alternative energy source, during early lactation (Herdt, 2000). Clinical ketosis was marked by loss of appetite, decreased milk production and a distinctive sweet acetone breath, while subclinical ketosis can be detected by elevated blood or milk ketone levels (Van Saun, 1997; Duffield, 2000).

Managing body condition scores (BCS) during mid to late lactation was crucial, as cows with higher BCS were more susceptible to fatty liver and ketosis (Kaneene and Miller, 1994; Herdt , 2000). Maintaining optimal BCS between 3.0 and 3.75 on a 5-point scale, was recommended to manage NEB and improve health and production during transition period (Duffield *et al.*, 1997; Van Saun, 1997). Negative energy balance, often caused by inadequate dry matter intake, has been associated with longer intervals to first ovulation and reduced fertility (Lucy *et al.*, 1992). Furthermore, high-protein diets can have mixed effects on reproductive outcomes; some research indicates that elevated urea nitrogen levels may disrupt reproductive tract secretions (Hutjens and Jordan, 1994). Successful management of this period required careful attention of nutrition and management. Hence, the present study focuses on the identification of certain factors that might affect the resumption of ovarian cyclicity of postpartum crossbred dairy cows.

Materials and methods

The study was conducted at Livestock Research Station (LRS), Thiruvazhamkunnu. Forty postpartum crossbred dairy cows belonging to the age group 2 to 10 years and parity 1- 6 with a BCS score ranging from 2.5 - 3.75 were selected for the study. Peripartum complications of the selected animals were recorded.

The BCS of the animals under study were assessed based on the fat disposition over certain body regions as per Smijisha (2012). Animals were examined per rectally at 15 days interval starting from day 45 to day 90 postpartum to check the ovarian changes including corpus luteum (CL).

Blood (5 mL) was collected from the jugular vein on days five and 45 postpartum to estimate the complete blood count using a fully automated haematological analyser (Mindray BC-30 Vet, Shenzhen Mindray Animal Medical Technology Co., Ltd., China). Serum macro minerals, including calcium (Ca), phosphorus (P) and magnesium (Mg), were estimated using the modified Arsenazo III method, phosphomolybdate methodology, and xylidyl blue method, respectively, with Austrian Genetics Dairy Biotech kits (Austria) in a semi-automatic analyser (Erba Chem 7, Germany). Blood was also collected at 15-day intervals, starting from day 30 to day 90 postpartum. Serum was separated by centrifugation (3000 rpm) and stored at -20°C until assayed for total protein (biuret method) and cholesterol (endpoint method) using Austrian Genetics Dairy Biotech kits (Austria) in a semi-automatic analyser (Erba Chem 7, Germany). Serum progesterone concentration from day 45 to day 90 postpartum, at 15-day intervals, was estimated by the radioimmunoassay (RIA) method using IM1188 Beckman Coulter Inc. RIA progesterone kits (France). Cows with serum progesterone level >1 ng/mL was considered as resumed ovarian cyclicity. Based on the progesterone level the animals studied were divided into three groups *viz.* early resumed (ROA within 60 days postpartum), late resumed (ROA from day 60 to day 90 postpartum) and not resumed cyclicity groups (progesterone value was < 1 ng/ mL during the entire study period).

Results and discussion

Out of 40 crossbred dairy cows selected 22 animals had early resumed cyclicity (by 60 days postpartum), seven animals late resumed (60-90 days postpartum) and 11 animals did not resume cyclicity until 90 days postpartum.

Serum progesterone concentration of cows that resumed cyclicity were significantly higher ($p<0.05$) than that of cows which did not resume cyclicity during post-partum (Table 1). Serum progesterone concentration ≥ 1 ng/ mL was indicative of the presence of CL and is considered as the resumption of ovarian cyclicity in post-partum crossbred cows under study. Results obtained were in agreement with the observations of Sonam *et al*. (2020), as they observed that serum progesterone levels were significantly higher in crossbred dairy cows that had resumed ovarian cyclicity postpartum compared to the animals which did not resume.

There was no significant difference ($p>0.05$) between the age of animals which resumed cyclicity early (5.09 ± 0.47) and late (5.14 ± 0.34) during postpartum (Table 2). Conversely, on regression analysis it was observed that cows aged ≤ 5 years showed significantly ($p<0.05$) higher chance for ROA during postpartum compared to animals aged >5 years (odds ratio- 0.066; Table 2). Cushman *et al*. (2010) observed that mature cows initially had more follicles which declined after age 6 years of age. Despite this reduction, ovarian size continued to increase, but the decrease in follicle count with advanced age was linked to higher chances of ovulation failure and lower pregnancy rate.

On regression analysis multiparous crossbred cows showed significantly ($p<0.05$) higher chance for ROA during postpartum compared to primiparous cows (odds ratio- 10.558) (Table 3). Tanaka *et al*. (2008), observed that primiparous cows had shown significantly ($p<0.05$) higher days from calving to first ovulation (31.8 ± 8.3 d) compared to multiparous cows (17.3 ± 6.3 d). Primiparous cows had a more unbalanced metabolic and endocrine profile than multiparous cows, suggesting they require more time to recover from the negative energy balance period. Similar observations were made by Santos *et al.* (2009) and Zhang *et al*. (2010). According to Meikle *et al*. (2004), multiparous cows tend to have more body reserves and better hormonal balance and recover more quickly from transitional stress than primiparous animals to resume cyclicity which could be the reason for the present finding.

Body condition score of early and late resumed cyclic cows were significantly higher ($p<0.01$) than those which did not resume cyclicity during postpartum period and their BCS were 3.16 ± 0.05, 3.36 ± 0.07 and 2.89 ± 0.09, respectively (Table 2). The results were comparable with the findings of Taylor *et al*. (2003) as they reported that cows with delayed ovulation had significant BCS loss that linked to prolonged NEB and reduced dry matter intake, while the higher BCS associated with early ROA during postpartum (2.9 ± 0.06 *vs*. 2.6 ± 0.07, p = 0.001). During the postpartum period, the energy requirement for peak milk yield surpasses the energy intake, resulting in negative energy balance (NEB) that lasts for 10 to 12 weeks postpartum (Sartori and Mollo, 2007). This condition is also associated with body condition score (BCS) loss, which is related to delayed ovulation and an increased number of days open (Butler and Smith, 1989; Ferguson, 1991; Zulu *et al*., 2002). Although there was no significant difference in milk production or BCS between cows that resumed cyclicity early and those that resumed later, early-resumed cows tended to produce more milk. This suggests that these cows may have used some of their body fat reserves to support higher milk production, which could explain why their BCS was slightly lower compared to the late-resumed cows, even though the difference was not statistically significant.

Milk production was significantly higher ($p < 0.01$) in cows that did not resume oestrous cycles during the postpartum period (15.82 ± 0.57 L) compared to cows that resumed cycles early (11.55 ± 0.49 L) or late (10.86 ± 1.14 L) (Table 2). The results were in accordance with the findings of Taylor *et al*. (2003), where the cows with higher milk yield showed reduced dry matter intake early in lactation, and appreciable BCS loss due to prolonged NEB and exhibited delayed ovulation. While some studies suggested that high milk production could delay the first postpartum ovulation by up to 14 days (Lucy, 2001; Gong *et al*., 2002).

Serum Ca concentration (day 5) in cows which resumed cyclicity was significantly($p<0.05$) higher (8.54 ± 0.33 *vs* 7.00 ± 0.32 mg/dL) than that of cows which did not resume cyclicity during early postpartum period (Table 4). Calcium is essential for maintaining uterine muscle tone and its deficiency can lead to delayed uterine involution and extended calving intervals (Bindari *et al*., 2013), which is consistent with findings of reduced blood Ca in postpartum anoestrus cows (Dutta *et al*., 2001; Das *et al*., 2012; Jayachandran *et al*., 2013). Ugyen and Dorji (2016) reported that postpartum cyclic crossbred cows had a significantly higher serum Ca concentration (10.32 ± 0.21 mg/dL) compared to postpartum anoestrous cows (9.61 ± 0.16 mg/dL, $p<0.05$). Cows that did not resume cyclicity had shown higher milk production as compared to cows that resumed cyclicity, which indicated that cows that did not resume cyclicity showed more energy partition into lactation than into reproduction. The higher milk production increases their calcium demand and this may lead to drain of calcium from their bones or boost absorption from the gut to meet the demand. This may be a reason for cows that did not resume cyclicity in the postpartum period showing higher serum calcium levels during day 45 postpartum as compared to cows that resumed cyclicity

Serum total protein ($p<0.01$) and cholesterol ($p<0.05$) concentration in cows that resumed cyclicity were significantly higher on day 75 postpartum, as compared to animals that did not show ROA postpartum (Table 5 and 6). The results are consistent with those of Uddin *et al*. (2019), who reported significantly higher serum total protein levels in cyclic cows (61.74 ± 3.96 g/L) compared to postpartum anestrous cows (45.73 ± 3.20 g/L). These findings are similar to those of Pariza *et al.* (2013) and Qureshi *et al*. (2016). Lower serum protein levels in anoestrus cows were associated with amino acid deficiencies for reproductive hormonal regulation, leading to ovarian dysfunctions (Tedeschi *et al*., 2015) and extended voluntary waiting periods (Veena *et al*., 2015). Borpujari *et al*. (2019), observed that serum cholesterol concentrations were significantly higher in normal cyclic postpartum cows (204.70 ± 7.40 mg/dL) compared to noncyclic cows (153.90 ± 8.25 mg/dL) on day 45 postpartum ($p<0.01$). These findings support the observations by Das *et al*. (2012), where normal cyclic cows had significantly higher serum cholesterol levels (65.31 ± 5.58 mg/dL) compared to anoestrus cows (50.06 ± 2.53 mg/dL, $p<0.05$). Cholesterol plays a key role in steroid hormone production because it acts as a building block for these hormones in ovarian cells (Rabiee and Lean, 2000).

In the present study serum P and Mg did not show any significant ($p>0.05$) association with ROA in postpartum dairy cows.

Table 1. Comparison of serum progesterone concentration (mean ± SE) of cows which resumed or not resumed ovarian cyclicity at different postpartum days

Postpartum Period (Days)	Progesterone concentration (ng/mL)		t-value	p-value
	Resumed (n=29)	Not resumed (n=11)		
Day 45	0.52 ± 0.10	0.63 ± 0.08	0.654	0.517^{ns}
Day 60	1.34^{a} ± 0.16	0.59^{b} ± 0.07	4.394	<0.001**
Day 75	1.00^{a} ± 0.22	0.38^{b} ± 0.07	2.736	0.010**
Day 90	1.42^{a} ± 0.22	0.50^{b} ± 0.06	4.101	<0.001**
F-value	2.352	0.597		
P-value	0.078^{ns}	0.561^{ns}		

*** Significant at 0.01 level; ns non-significant*

Table 2. Comparison of age, BCS and milk production (mean ± SE) with resumption of cyclicity at different postpartum days in study population

ROA	Animal factors		
	Age (years)	BCS (scale 1-5)	Milk Production (L)
Not resumed (n=11)	4.36 ± 0.54	2.89^{b} ± 0.09	15.82^{a} ± 0.57
Early resumed(n=22)	5.09 ± 0.47	3.16^{a} ± 0.05	11.55^{b} ± 0.49
Late resumed(n=7)	5.14 ± 0.34	3.36^{a} ± 0.07	10.86^{b} ± 1.14
F-value	0.584	8.105	14.625
P-value	0.563^{ns}	0.001**	<0.001**

*** Significant at 0.01 level; ns non-significant; Means having different letter as superscript differ significantly within in a column*

Table 3. Logistic regression analysis to identify the risk factors of resumption of ovarian function

Variable	Classification	n (%)	p-value	Odds ratio	95% CL
Age	≤ 5 years	26 (65.0)	0.049*	0.066	0.004 – 0.985
	>5 years	14 (35.0)			
Parity	Primiparous	10 (25.0)	0.043*	10.558	1.072 – 104.01
	Multiparous	30 (75.0)			

Table 4. Comparison of serum levels of calcium, phosphorus and magnesium (mean ± SE) of cows which resumed or not resumed ovarian cyclicity at different postpartum days

Serum macro-minerals (mg/dL)	Days postpartum	Resumed (n=29)	Not resumed (n=11)	t-value	p value
Calcium	Day 5	8.54^{a} ± 0.33	7.00^{b} ± 0.32	2.689	0.01*
	Day 45	8.42 ± 0.33	9.55^{a} ± 0.52	1.793	0.081^{ns}
	t-value	0.199	4.252		
	p-value	0.844	0.002**		
Phosphorus	Day 5	4.65 ± 0.22	4.86 ± 0.33	0.516	0.609^{ns}
	Day 45	5.06 ± 0.25	4.54 ± 0.51	1.019	0.315^{ns}
	t-value	1.430	0.483		
	p-value	0.164	0.639^{ns}		
Magnesium	Day 5	3.1 ± 0.16	3.42 ± 0.23	1.058	0.297^{ns}
	Day 45	2.9 ± 0.13	2.9 ± 0.21	0.002	0.998^{ns}
	t-value	1.238	1.313		
	p-value	0.226^{ns}	0.218^{ns}		

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

Table 5. Comparison of total protein levels (mean ± SE) of cows which resumed or not resumed ovarian cyclicity at different postpartum days

Postpartum Period (Days)	Total protein (g/dL)		t-value	p-value
	Resumed (n=29)	Not resumed (n=11)		
Day 30	6.66 ± 0.24	6.77 ± 0.44	0.231	0.819^{ns}
Day 45	6.48 ± 0.25	5.6 ± 0.56	1.674	0.102^{ns}
Day 60	6.85 ± 0.40	6.06 ± 0.59	1.059	0.296^{ns}
Day 75	7.26^{a} ± 0.31	5.48^{b} ± 0.57	2.921	0.006^{**}
Day 90	5.86 ± 0.41	5.25 ± 0.53	0.811	0.422^{ns}
F-value	2.437	0.807		
p-value	0.081^{ns}	0.528^{ns}		

*** Significant at 0.01 level; ns non-significant*

Table 6. Comparison of cholesterol levels (mean ± SE) (mg/dL) of cows which resumed or not resumed ovarian cyclicity at different postpartum days

Postpartum Period (Days)	Serum cholesterol level (mg/dL)		t-value	p-value
	Resumed (n=29)	Not resumed (n=11)		
Day 30	111.84^{c} ± 4.92	98.02 ± 10.88	1.331	0.191^{ns}
Day 45	119.43^{bc} ± 4.44	109.99 ± 11.2	0.952	0.347^{ns}
Day 60	126.67^{abc} ± 7.22	110.36 ± 11.7	1.185	0.243^{ns}
Day 75	143.88^{a} ± 8.71	110.97 ± 12.16	2.053	0.047**
Day 90	131.23^{ab} ± 7.18	123.04 ± 13.67	0.571	0.572^{ns}
F-value	3.826	0.807		
p-value	0.006**	0.528^{ns}		

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

Means having different letter as superscript differ significantly within in a column

The resumption of ovarian cycles depended on balancing energy and nutrient allocation between lactation and reproduction. During early lactation, essential nutrients were diverted to support milk production, which could delay the reinitiation of oestrous cycles (Stevenson *et al*., 1997). Many animal factors such as age, parity, BCS determined by NEB could delay the ROA. However, the influence of many micro-minerals and blood metabolites at subcellular level altering various metabolic pathways during transition period need to be elucidated to derive valuable conclusions.

Conclusion

The current data revealed that ROA during postpartum was influenced by age, parity, BCS, milk yield, serum Ca, total protein, and cholesterol concentrations. Higher parity, age > 5years and BCS showed a positive effect on ROA. The animals with higher milk yield exhibited a delayed ROA during the study. Moreover, hypocalcaemia delayed ROA and higher concentrations of total protein and cholesterol enhanced ROA. In this study no influence of P and Mg could be noticed on ROA during postpartum in crossbred dairy cows. This might be due to the proper feeding and mineral supplementation maintained in the farm.

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