RESEARCH ARTICLE

Influence of Blood Metabolites on Ovarian Rebound in Postpartum Sahiwal Cows

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Abstract

Present investigation was carried out to study the influence of blood metabolites, *viz*., glucose and NEFA on ovarian rebound in postpartum Sahiwal cows. Advance pregnant Sahiwal cows (n=14) maintained at Bull Mother Experimental Farm at Anjora, Durg (CG), India were selected and divided into two Groups, Group I comprised of animals (n=7) with BCS ≥ 2.75 and that of Group II comprised of animals (n=7) with average BCS < 2.75 at one week before parturition. Trans-rectal ultrasound scanning of ovaries was accomplished in animals of both groups on alternate days from 21 to 51 days postpartum to record the development of dominant follicles (DF). Animals of Group-I without DF recorded higher pre-partum BCS than those with DF (n=3). Non-significant differences were recorded in mean serum glucose concentration between animals of Group-I and Group-II during all days of observation. Significant differences were (p<0.01) observed in mean serum glucose concentration between animals with and without DF of Group-I on day 20 postpartum (39.79±0.56 vs 43.53±0.29 mg/dl). Animals (n=3) with DF of Group-I showed significantly increasing trend of mean serum glucose level from 20 to 50 days of postpartum, whereas the animals (n=4) without DF recorded similar serum glucose levels between 10 and 40 days postpartum. Significantly (p < 0.05) higher mean serum NEFA concentrations were recorded in animals without DF than in animals with DF of Group-I on all days from the day of calving to 50 days postpartum, except on day 10. It may be concluded that higher serum NEFA concentration influenced the development of dominant follicle in postpartum Sahiwal cows.

Keywords: BCS, Dominant follicle, Glucose, NEFA, Ovarian rebound, Sahiwal cows.

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Introduction

The duration of postpartum period has an important influence on reproductive performance of dairy cows (Peter *et al*., 2009). It is well accepted fact that the onset of normal ovarian cyclicity is one of the key events in dairy cows to maintain their maximum breeding potential following parturition. Some reports have been published on the relationships among the resumption of postpartum ovarian cyclicity and nutritional end points, such as body condition score (BCS), body weight and several plasma metabolites in high-producing dairy cows (Pushpakumara *et al*., 2003; Reist *et al*., 2003). High-yielding dairy cows need more energy for milk production during transition from late pregnancy to early lactation and undergo negative energy balance (NEB), which leads to the mobilization of fatty acids from adipose tissues (Zhang *et al*., 2019). Adipose tissue plays important role in the dynamic control of energy metabolism and adequately regulated lipolysis is necessary for dairy cows to successfully adapt to NEB, and the limited release of non-esterified fatty acid (NEFA) can fully meet the energy demand (Bradford *et al*., 2015; Contreras *et al*., 2017). Concentration of NEFA in blood is a good indicator of adipose tissue mobilization.

NEB during early lactation is the major nutritional link to low fertility in lactating dairy cows. NEB delays recovery of postpartum reproductive function and exerts carryover effects that reduce fertility during the breeding period. NEB acting perhaps through the combined metabolic signaling of low blood glucose and insulin concentrations along with elevated NEFA, delays the rise in gonadotropin (LH and FSH) pulses necessary for stimulation of ovarian follicular development. The delay to the beginning of energy balance recovery after parturition is directly correlated with the delay from parturition to first ovulation (Kadokawa and Martin, 2006). The follicular growth can be detected as early

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as about 4 to 5 days postpartum, and the ovarian changes occur every 4 days during the postpartum period in dairy cows. Moreover, the average number of small follicles (3 to 5 mm diameter) is decreased by day 25 postpartum and the number of large follicles (10 to 15 mm or >5 mm) is increased with increasing postpartum days (Lucy *et al*., 1991). With the above perspectives, the objective of present study was to investigate the influence of blood metabolites, *viz*., glucose and NEFA and BCS on ovarian rebound after calving in Sahiwal cows.

Materials and Methods

The present investigation was carried out on 14 advance pregnant Sahiwal cows maintained at Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry Anjora, Durg (CG), India. The experiment was conducted for a period extending from one week before due date of parturition to 51 days postpartum. The animals were divided into two groups, Group-I was comprised of animals (n=7) with good body condition score (BCS) ≥ 2.75 and Group-II consisted of animals (n=7) with average BCS <2.75 at one week before parturition.

BCS in the scale of 1-5 with 0.25 increments were recorded in animals at one week before due date of calving, on the day of calving and at 10, 20, 30, 40 and 50 days postpartum. Blood samples were collected from all the animals on each day of BCS observation. The serum samples separated out by centrifugation were stored at –20ºC in a deep freeze until further analysis. Serum glucose level was estimated by Glucose Oxidase-Peroxidase reagent (GOD/ POD method) through Biolab Diagnostics Kit and value was expressed in mg/dl. Serum non-esterified fatty acids (NEFA) level was determined by modified soap extraction method and value was expressed in mM/ml.

Postpartum ovarian activity was monitored using a Real-time B-mode ultrasound scanner (Prosound ALOKA Japan) equipped with a convertible 5.0-7.5 MHz linear array transducer designed for intra-rectal placement. Postpartum ovarian activity was monitored on alternate days beginning from day 21 postpartum till appearance of first postpartum dominant and/ or ovulatory follicle (≥ 10 mm in diameter) or up to day 51 postpartum. The mean and variances were calculated for each variable in animals and difference of significance was determined by independent 't' test. One-way ANOVA was applied and difference within group and between days was tested by Duncan's multiple range test using SPSS computer programme version 16.

Results and Discussion

Postpartum Ovarian Activity

In the present study, the ovarian activity was monitored through ultrasound scanning on alternate day from day 21 to day 51 postpartum and the follicular population was recorded with respect to their size in terms of diameter. The ovaries were characterized by growth and regression of several small (up to 5 mm), medium follicle (5-7 mm diameter) and large follicle (> 7 - < 10 mm diameter) and with the detection of first postpartum dominant follicle (≥ 10 mm) in few animals. Three animals of Group-I (3/7, 42.85%) showed presence of dominant follicle (≥ 10 mm) between days 31 and 51 postpartum, while remaining 4 animals of Group-I (57.14%) and all 7 animals of Group-II (BCS pre-partum <2.75) did not show presence of dominant follicle during the study period.

Blood Metabolites Profile

Mean (±SE) serum glucose and NEFA concentrations in animals of both groups are presented in Table 1. Mean serum glucose concentration in animals of two groups ranged between 40.82 and 60.51 mg/dl and that of NEFA concentration between 0.45 to 1.35 mM/ml from 7 days pre-partum to 50 days postpartum.

Highly significant increase in concentration of serum glucose was recorded in animals of both the groups on the day of calving, which may be due to higher demand of energy for synthesis of colostrum and parturition process. However, thereafter the serum glucose concentration showed reducing trend up to 20 days postpartum in animals of both the groups, which may be due to energy required for milk production

Table 1: Mean ± SE of serum glucose and NEFA concentrations in postpartum Sahiwal cows with different BCS pre-partum

	Serum glucose (mg/dl)		*Serum NEFA (mM/ml)*	
Days postpartum	*Group-I BCS ≥ 2.75 (N=7)*	*Group-II BCS < 2.75 (N=7)*	*Group-I BCS ≥ 2.75 (N=7)*	*Group-II BCS < 2.75 (N=7)*
–7	46.56 ± 0.78^{c}	45.26 ± 0.12^{b}	0.49 ± 0.01^{a}	0.45 ± 0.00^{a}
0	60.51 ± 0.58^{d}	58.31 ± 1.01^{c}	$1.35 \pm 0.07^{**d}$	0.97 ± 0.00^{e}
10	42.95 ± 0.35^{b}	42.98 ± 0.34^{b}	$1.01 \pm 0.02^{**c}$	0.86 ± 0.01^{d}
20	40.89 ± 0.46^{a}	40.82 ± 0.62^{a}	$0.91 \pm 0.02^{*bc}$	0.84 ± 0.01^{d}
30	42.99 ± 0.47^{a}	43.08 ± 0.57^{b}	$0.87 \pm 0.02^{*b}$	0.80 ± 0.01^{c}
40	44.13 ± 0.48^{b}	44.09 ± 0.37^{b}	$0.84 \pm 0.02^{*b}$	0.78 ± 0.01^{bc}
50	46.32 ± 0.44^{c}	45.12 ± 0.52^{b}	0.81 ± 0.02^{b}	0.75 ± 0.01^{b}

Means bearing different superscripts within a column differ significantly ($p < 0.05$).
*$p < 0.05$; **$p < 0.01$) between columns within days.

Table 2: Mean ± SE of serum glucose and NEFA concentration in postpartum Sahiwal cows of Group-I (BCS ≥2.75) with and without dominant follicles

	Mean ± SE serum glucose concentration (mg/dl)		*Mean ± SE serum NEFA concentration (mM/ml)*	
Days postpartum	*Animals with DF (n=3)*	*Animals without DF (n=4)*	*Animals with DF (n=3)*	*Animals without DF (n=4)*
−7	46.38 ± 0.63^{c}	46.70 ± 1.39^{b}	0.50 ± 0.02^{a}	0.48 ± 0.02^{a}
0	59.95 ± 0.82^{d}	60.94 ± 0.84^{c}	1.09 ± 0.01^{e}	$1.14 \pm 0.01^{*d}$
10	42.19 ± 0.44^{b}	$43.53 \pm 0.29^{*a}$	0.89 ± 0.02^{d}	0.99 ± 0.03^{c}
20	39.79 ± 0.56^{a}	$43.53 \pm 0.29^{**a}$	0.84 ± 0.00^{c}	$0.94 \pm 0.02^{*bc}$
30	42.30 ± 0.75^{b}	43.51 ± 0.55^{a}	0.81 ± 0.01^{c}	$0.91 \pm 0.02^{*bc}$
40	44.69 ± 0.72^{c}	43.70 ± 0.64^{a}	0.79 ± 0.00^{bc}	$0.88 \pm 0.02^{*b}$
50	46.11 ± 0.78^{c}	46.47 ± 0.61^{b}	0.76 ± 0.02^{b}	$0.89 \pm 0.05^{*b}$

* $p < 0.05$; ** $p < 0.01$) between columns within days.
Means bearing different superscripts within the column differ significantly ($p < 0.05$).

coupled with reduced feed intake forcing the transition cows to undergo negative energy balance (NEB). The level of serum glucose concentration increased consistently from days 20 to 50 postpartum in animals of both groups. It is interesting to note that despite significant difference in BCS between animals of two groups, they did not show difference in serum glucose level on any day of observation.

Findings of present study concurred with the earlier report of significant increase in glucose concentration with approaching parturition, which was followed by reduction in concentration up to 22 days after parturition (Rawat *et al*., 2006). The levels of serum glucose concentration rose in animals of both the groups from 30 to 50 days postpartum. McGuire *et al*. (2004) and Moallem *et al*. (2000) reported return to positive energy balance between 4 and 5 weeks after calving, while other researcher reported longer duration of 6 to 8 weeks to return positive energy balance (Carson *et al*., 2007) and that as the lactation advanced the glucose levels rose consistently, which approximated with the finding of present study.

Serum NEFA concentration was the maximum in animals of both the groups on the day of calving (Table 1), which might be due to mobilization of reserve body fat using it as source of energy to fulfil higher demand for synthesis of colostrum and process of parturition. This mobilization of reserve body fat resulted in sudden reduction in BCS from pre-calving level. Significantly higher ($p<0.01$) mean serum NEFA concentration was observed in cows of Group-I than that of Group-II (1.35±0.07 vs 0.97±0.00 mM/ml) on the day of calving indicating that the rate of mobilization of reserve body fat is greater in animals with higher BCS at pre-calving stage than in animals with less BCS at similar stage. Thereafter, a similar trend of continued reduction in serum NEFA concentration was recorded from day of calving up to 50 days postpartum in the animals of both the groups; however, its level did not reach to pre-calving status till 50 days postpartum. It is interesting to note that animals with higher BCS recorded significantly higher NEFA concentration on each day of observation than in animals with less BCS, suggesting greater rate of mobilization of reserve body fat in animals with higher BCS.

Effect of Serum Metabolites on Postpartum Ovarian Activity

Mean (± SE) serum glucose and NEFA concentration in postpartum Sahiwal cows of Group-I with and without dominant follicles (DF) is presented in Table 2. The animals with dominant follicles (n=3) showed significantly increasing trend of mean serum glucose level from 20 to 50 days of postpartum.

Although the animals without DF (n=4) recorded significantly higher mean serum glucose concentration on days 10 and 20 postpartum than in animals with dominant follicles, but its level remained static between 10 and 40 days postpartum. First postpartum dominant follicles was observed between 30 and 50 days of postpartum in three animals corresponding to the period when level of serum glucose showed increasing trend suggesting its role in growth and development of ovarian follicle.

Hypoglycaemia has been shown to have a negative impact on the process of resumption of the follicular growth during postpartum period (Lucy, 2016). In that respect, it has been reported that glucose together with insulin is the most likely molecule that exerts an effect on GnRH secretion in postpartum dairy cows (Leroy *et al*., 2008). The circulating level of IGF-1 is affected by insulin concentration in blood which in turn depends on circulating level of glucose. Therefore, circulating levels of insulin and IGF-1 starts to increase with the increasing concentrations of glucose. IGF-1 has been shown to represent a 'metabolic signal' of the resumption of ovarian function (Stamples *et al*., 1990). A decreasing trend was observed in serum NEFA concentration from day of calving to 50 days postpartum in animals with and without dominant follicles (Table 2). However, the animals without dominant follicles recorded significantly higher ($p< 0.05$) mean serum NEFA concentration on day of calving and from 20 to 50 days postpartum than in animals with dominant follicles.

The findings of the present study suggest close relationship between development of dominant follicles and decreasing pattern of serum NEFA concentration with rising trend of serum glucose concentration during early postpartum period. High NEFA concentrations have a direct toxic effect on the ovaries, and more specifically, a direct toxic effect of high NEFA levels on oocyte developmental competence, and also on granulosa cells has been documented (Kruip and Kemp, 1999). Concentrations of NEFA in plasma and follicular fluid are closely related, and a negative relationship between follicular concentrations of NEFA and estradiol has been demonstrated (Comin *et al.,* 2002; Jorritsma *et al.*, 2004). There are many changes in the metabolic milieu associated with negative energy balance in the peri-parturient cows that are implicated in reduced reproductive performance. Pulsatile release of LH is diminished, circulating levels of insulin, IGF-I, leptin and glucose are depressed; and non-esterified fatty acids and growth hormone are elevated (Butler, 2003; Diskin *et al.*, 2003).

From the findings, it may be concluded that development of dominant follicle is affected by circulating levels of glucose and NEFA during peri-parturient period in Sahiwal cows and decreasing concentration of serum NEFA coupled with increasing levels of serum glucose is associated with growth and development of ovarian follicle during early postpartum period.

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