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Some Metabolic Parameters During Transition Period in Dairy Cows with and without Retained Fetal Membranes

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ABSTRACT

This study evaluated the levels of some serum metabolites for dairy cows (Holstein Friesian) in relation to expulsion of fetal membranes. Blood concentrations of glucose, triglycerides, total cholesterol and non-esterified fatty acid (NEFA) were determined (from 7 days before, day of parturition and 2 days after calving) in cows that expelled fetal membranes normally (n = 9 cows) and in cows that developed retention of fetal membranes (RFMs), n = 17). The cows that developed RFMs had lower concentrations of cholesterol at and after parturition, while the concentrations of NEFA and triglycerides were higher than those in cows that normally expelled the fetal membranes. Results revealed that the concentration of NEFA before calving was highly related to the RFMs. It may be inferred that monitoring the levels of NEFA during the prepartum period would help in identifying the cows at the risk of developing RFMs.

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Introduction

The transition period (from 3 weeks before to 3 weeks after calving) is critically important to health, production, and profitability of dairy cows. Most health disorders occur during this time (Drackley, 1999). One of the most common problems seen in dairy cattle are retained fetal membranes (RFMs) (Smith and Risco, 2005; Seifi *et al.*, 2007) and it is an economically important disorder that affects dairy cows in puerperal period (Laven and Peters 1996; Kelton, *et al.*, 1998). Normal expulsion of fetal membranes occurs within 8-12 hours after calving. RFMs are defined as failure to expel fetal membranes within 24 hours (Kelton *et al.*, 1998). Both the mechanism of placenta expulsion and the cause of RFMs remain unclear (Seifi *et al.*, 2007). Early detection of cows at increased risk of developing RFMs could improve treatment successes and result in improved milk production and reproductive performances (Huzzey *et al.*, 2011; Bjerre-Harpøth *et al.*, 2012).

In recent years, multiple physical, endocrine and cellular factors are involved in expulsion of fetal membranes. These include uterine atony, edema of the chorionic villi, cellular dysfunction and necrosis, incomplete breakdown of extracellular matrix, decrease innate and humeral immune responses and oxidative damage (Drillich, 2001; McNaughton and Murray, 2009). Various risk factors such as parity, hereditary, environment, hormones, nutrition, metabolic parameters (e.g. energy, lipid, and protein status related molecules e.g. glucose, non-esterified fatty acids (NEFAs), b-hydroxy butyric acid (BHBA), cholesterol, triglycerides and urea nitrogen, etc..) and oxidative status related molecules have been suggested as a cause of RFMs (Hayirli *et al.*, 1998; Seifi *et al.*, 2007; Huzzey *et al.*, 2011; Bjerre-Harpøth *et al* 2012). The alteration in energy, lipid and protein status related molecules had been reported during the transition period by Seifi *et al.* (2007) and Qu *et al.* (2014).

The peripartum period is marked by reduced dry matter intake (DMI), leading to negative energy balance (NEB) (Hayirli *et al.* 1998). During NEB, mobilization of body fat and protein reserves occurs to achieve the energy requirements (Drillich, 2001). The reduction in the DMI and changes in energy demand with resultant lipid mobilization increases NEFA and BHBA (Grum *et al.*, 1996; Reist *et al.*, 2002). Several studies have reported a clear variation of blood energy, lipid, and protein status related molecules during the transition period (Kaczmarowski *et al.*, 2006; Seifi *et al.*, 2007; Quiroz-Rocha *et al.*, 2009; Civelek *et al.*, 2011). Moreover, elevated NEFA concentrations in last week pre- parturition are risk indicators for

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different diseases in early lactation period in dairy cows (Ospina *et al*., 2010; Moyes *et al*. 2013). Thus, the assessment of metabolites such as glucose, triglycerides, cholesterol and NEFA may provide clues concerning the risk of development of RFMs under our environmental condition.

The objective of this study was to compare the levels of some metabolites during peripartal period, from 7 days before to 7 days postpartum and between dairy cows that developed RFMs and those that remained healthy during the postpartum period (Non-RFMs).

Materials and methods

Animals

The study was conducted on dairy farm that belongs to El-Deabat, Sohag Governorate, Egypt. The herd consisted of approximately 500 lactating (Holstein Friesian) cows with daily milk yield that was ranged from 18–25 Kg. The cows were housed in an open shed with concrete floor and fed during winter on Barseem, rice straw and concentrates mixture, while during summer season, animals were fed on Drawa instead of Barseem. Pregnant cows were shifted to maternity pen a three weeks before expected date of calving and retained till about two weeks after calving. The nutrient requirements of these pregnant cows were met with adlib green fodder and concentrate.

A total of 30 pregnant dairy cows were initially enrolled for this study from November 2017 to March 2018. The selected cows for this study were within the range of 2–6 parity, BCS was ranged from 2.5–4 (1= emaciated and 5 = obese) according to (Spitzer, 1986). Out of them, four cows were excluded from study due to death, dystocia and paraplegia. Because of the difficulty in predicting definite date of calving, blood samples were taken prior to calving every 2-3 days by about three weeks and grouped into category of days 7 to 2 prepartum, then, samples were collected at day of calving (day 1) and days 2 to 7 post calving.

Examination of animals

Cows with or without RFMs were detected using visual and vaginal examinations, within 24 hours after calving. Seventeen dairy cows that not expelled fetal membranes within ≥24 hours after calving were grouped as RFMs group and nine dairy cows that were clinically healthy and expelled fetal membranes within 8 to < 24 hours were grouped as Non-RFMs and constituted the control group.

The study was performed according to the ethics and regulations of research studies and approved by ethical committee at Sohag University.

Samples

Blood samples were collected from the jugular vein of dairy cows (n.=26) early in the morning. Two types of blood samples were collected; whole blood samples that were collected on Vacutainer tubes containing sodium fluoride as anticoagulant and used for measuring of glucose level. Another blood sample was collected on plain Vacutainer tube and used for separation of serum for biochemical analysis. Plasma and serum samples were separated according to Coles (1986), and then stored at -20ºC till analysis.

Biochemical analysis

Commercially available kits were used to measure plasma concentration of glucose (Spectrum Diagnostics, Cairo), Serum concentrations of triglycerides (Vitro Scient, Cairo), cholesterol L-Q (Spinreact, Spain) and NEFA (Randox Laboratories LTD, UK).

Statistical analysis

Statistical analysis was done according to general linear model (G.L.M) of S.A.S program (2001), Version 8.2. Differences between groups for glucose, triglycerides, cholesterol and NEFA were evaluated by one-way ANOVA. Duncan Multiple Range Test (Steel and Torrie, 1980) was used to test the effect of treatments. The data were presented as mean±S.E. Level of significance was set at $P<0.05$.

Results

Results revealed significant decrease in glucose, triglycerides and cholesterol levels during the partum and postpartum periods, when compared with the prepartum period in both groups. In NRFMs group, there was a significant decrease in NEFA level during parturition only. However, in RFMs group, NEFA level was significantly increased during the postpartum period (Table 1).

Discussion

Although glucose is the primary metabolic fuel and it is absolutely required for vital organ function, fetal growth, and milk production (LeBlanc, 2006). Glucose is an insensitive measure of energy status because it could be subjected to

Table 1. Concentration of glucose, triglycerides, cholesterol and NEFA in cows with NRFMs and RFMs during the transition period.

	Period	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	NEFA (mmol/l)
NRFMs	Prepartum	48.67 ± 1.33^{a}	26.56 ± 1.13^{a}	159.33 ± 0.71^{a}	1.25 ± 0.09^{a}
	Partum	40.77 ± 1.16^{b}	22.89 ± 0.65^{b}	147.22 ± 0.76^{b}	0.74 ± 0.04^{b}
	Postpartum	37.78 ± 0.95^{b}	21.22 ± 0.54^{b}	142.56 ± 1.06^{c}	1.13 ± 0.11^{a}
	P-Value	0.001	0.001	0.001	0.001
RFMs	Prepartum	48.29 ± 2.30^{a}	38.29 ± 1.88^{a}	160.18 ± 3.33^{a}	1.67 ± 0.06^{b}
	Partum	38.66 ± 1.38^{b}	30.47 ± 0.77^{b}	134.23 ± 1.19^{b}	1.53 ± 0.08^{b}
	Postpartum	38.84 ± 1.70^{b}	21.76 ± 0.89^{c}	131.53 ± 3.38^{b}	2.00 ± 0.07^{a}
	P-Value	0.001	0.001	0.001	0.001

Data were expressed as Mean±SE

In each column, values followed by different superscript is significant $(P<0.05)$

tight homeostatic regulation, but together with NEFA, it provides further insights into the adaptation to NEB (Herdt, 2000).

In this study we found that glucose level in blood of retained placenta (RP) cows was slightly (Insignificantly) lower during the peripartum period than that in healthy ones. This study also showed lower postpartum levels of glucose, which agreed with the findings of Sevinc *et al.* (2003) and Civelek *et al.* (2006). The decrease in glucose concentration during late gestation may result from fetal growth and mammary gland development (Guo *et al.*, 2007). In a previous study, Markiewicz *et al.* (2001) found that dairy cows with increased NEFA level, decreased glucose level during the last month of pregnancy is a predisposing factor for development of RP in the postpartum. In contrast, Kuzma *et al.* (1996) found that glucose level in blood of RP cows was higher during the peripartum period than that in healthy ones. These investigations indicated that energy metabolism imbalance and postpartum NEB may contribute to the development of RP. Serum levels of NEFA may be more useful to evaluate the postpartum metabolic status of the cows and energy imbalance that might predispose them to RP.

In the present study, we found higher triglycerides levels in RP cows (in prepartum, partum and postpartum periods) compared with the control group. The higher concentrations of triglycerides in RP cows might have been resulted from more energy needs (Seifi *et al.*, 2007).

Serum cholesterol levels have potential as indicators of disease risk in dairy cows (Kaneene *et al.*, 1993). In this Study, cholesterol level increased very slightly directly in the prepartum then it was below average reference values in cows with RP. Cows with RFMs had significantly ($P < 0.05$) lower blood total cholesterol concentrations on day of parturition and day 1 of calving than did cows that expelled fetal membranes normally. Macak *et al.* (1999) produced similar data in RP cows. Semacan and Sevinc (2005) had also reported that serum levels of cholesterol in cows with RP were lower than the control cows. Changes in serum cholesterol concentrations may be simply due to decrease of feed intake, because most cholesterol in ruminants is of intestinal origin (Uchide *et al.*, 1997).

NEFA concentrations were significantly ($P<0.05$) higher at prepartum, day of calving, and days 1 and 2 after calving in cows affected with RFMs than in cows that expelled the fetal membranes normally. The data presented here showed that variations in serum NEFA levels are important in cows with RP. This may be attributed to NEB during prepartum period, which continue after calving due to substantial increase in energy requirements for fetal development and the needs of colostrum synthesis and limited feed intake (Grummer *et al.*, 2010), which result in, release of fatty acids from adipose tissue that circulate as NEFA. The mobilized NEFA serve as an alternative energy source for non-mammary tissues (adipose tissue and muscles) to preserve glucose and can be used to synthesize milk fat (Leroy *et al.*, 2008). Cows in negative energy balance are more likely to develop clinical problems like ketosis, mastitis, retained placenta than cows in positive energy balance (Mulligan and Doherty, 2008).

Metabolic events associated with energy insufficiency, increased fat mobilization (Kaneene *et al.*, 1993) and NEB at the end of gestation (Sevinc *et al.*, 2003; Seifi *et al.*, 2007) are frequently related to increased risk of RP (Kaneene *et al.*, 1993; Seifi *et al.*, 2007). Excessive lipid mobilization due to NEB causes change of serum NEFA levels (Seifi *et al.*, 2007; Laszlo *et al.*, 2009). In the current study, NEFA concentration was significantly increased ($p<0.001$) one week prepartum in RP group in comparison with the control group.

It was reported that prepartum NEFA (Laszlo *et al.*, 2009; Ospina *et al.*, 2010) was significantly associated with the development of clinical diseases in dairy cows including RP, displaced abomasum, ketosis (Ospina *et al.*, 2010) and metritis (Hammon *et al.*, 1993). Seifi *et al.* (2007) concluded that concentration of NEFA was higher in cows with RP than cows without RP. Furthermore, the increased prepartum NEFA had also been found to increase the risk of RP (Ospina *et al.*, 2010; Chapinal *et al.*, 2011; Nogalski *et al.*, 2012).

In several smaller studies, no differences in prepartum NEFA concentrations were observed between cows with or without RP (Quiroz-Rocha *et al.*, 2009; Seifi *et al.*, 2011). Elevated NEFA is in all probability suitable metabolic indicator for characterizing the increased risk of RP (Laszlo *et al.*, 2009). Leblanc *et al.* (2005) similarly found that evaluation of NEFA was more strategic to monitor transition dairy cows. Serum levels of NEFA may be more useful to identify cows with a metabolic abnormality or energy imbalance that might predispose them to RP. (Quiroz-Rocha *et al.*, 2009). Ospina *et al.* (2010) observed that postpartum NEFA concentration was mostly associated with developing of RP. The present study showed a high postpartum level of NEFA in cows with RP. These results might be associated with postpartum NEB (Seifi *et al.* 2007), disrupted energy metabolism and adaptation problems.

Conclusion

From this study, it can be concluded that NEFA and triglycerides concentrations increased during the peripartum period in dairy cows that developed RFM, compared with the cows that expelled fetal membranes normally. High magnitude of NEB during the prepartum period could be the possible reason for RFM in these animals.

Conflict of Interests

The author(s) declared no conflict of interests exist.

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