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First Postpartum Ovulation and its Relation to Serum Concentration of Ketone Bodies, Triglycerides and Fatty Acid Composition in Periparturient Buffalo-Cows

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Abstract: The purpose of this study was to determine some chemical metabolites (Ketone bodies, triglycerides and some fatty acids) in buffaloes in relation to first post partum ovulation. Ten multiparous buffaloes cows reared in Animal Reproduction Research Institute were classified in two groups early resumed to ovarian activity (ER) and late resumed ovarian activity (LR) groups. The experimental period started one week pre-partum and lasted for the 5th week postpartum Both groups were subjected to the same managerial conditions and fed on the same ration. Ovaries were weekly examined by ultrasonography from 8 days postpartum till first ovulation. Body condition score and milk yield were determined. Blood samples were weekly collected to characterize the metabolic profiles. The results indicated no significant difference in body condition score between both groups. Milk yield increased ($P<0.05$) in LR group as compared with ER group. Changes in metabolic traits in early lactation expressed intensive lipolyses in LR group. Ketone bodies increased ($P < 0.05$) in LR than ER group at -1, 2, 4 and 5 weeks post calving. Serum B-hydroxybutyrate was increased ($P < 0.05$) in LR group at -1, 1, 4 and 5 weeks post calving. Serum acetone increased sharply after calving in LR than ER group. Serum triglycerides were decreased ($P < 0.05$) in LR than ER group. Long chain fatty acids indicated significant increase of palmetic, stearic and oleic acid in LR than ER group. Changes in linoleic and linolenic fatty acids are unclear. It was concluded that the values of ketone bodies triglycerides and fatty acids seem to affect the onset of postpartum ovarian cyclicity.

Key words: First ovulation · Ketone bodies · Fatty acids · Buffaloes · Calving · Postpartum period

INTRODUCTION

It is economically important for the buffalo breeders to have a successful breeding season, whereas, resumption of post partum ovulation was reported to affect the reproductive performance of buffaloes [1, 2]. In the starting phase of lactation (especially in the first 3-5 weeks after parturition and especially in high producing cows and buffaloes), feed intake is insufficient to cover the requirement of energy for maintenance of body tissue function and milk production, hence tissue reserves are utilized [3-6]. This has negative impact on days of first ovulation [7] and is associated with reduced post partum concentration of GnRH and LH that may adversely influence follicular development [8-10]. In case of excessive fat mobilization, the tricarboxylic acid cycle cannot fully metabolize fatty acids, consequently, acetyl-coenzyme-A is converted to acetoacetate (AcAc) which is then reduced to β-hydroxybuterate (βHBA) or spontaneously decarboxylized to actone (Ac). So, negative energy balance contributes to increase ketogenesis [11] which lead to some adverse consequences such as reduce milk production [12, 13] delay onset of ovarian cyclicity [14] and increase risk of devolving cystic ovaries [15]. Intensive lipolysis leads to high levels of non-esterified fatty acids accompanied by impairment of the reproductive performance [16]. In particular, the long chain fatty acids of phospholipid and stored triacylglycerols determine the fluidity of cell membrane and affects cell function [17-19]. Polyunsaturated fatty acids may affect metabolism via interactions with nuclear transcription factors [20] by altering production of prostaglandins and leukotriens [21] and by affecting cell signaling and signal transduction mechanisms [22, 23].Changes in serum metabolities during the periparturient period were important factors affect the resumption of ovulation. Based on that, the objectives of present study were to (1) determine the concentrations of serum ketone bodies, the individual one (Ac and βHBA), triglycerides and some

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long-chain fatty acids and their relation to the onset of post partum ovarian cyclicity; (2) to determine which metabolite (serum ketone bodies, βHBA or Ac) is more representative for the energy balance and consequently early detection of subclinical ketosis.

MATERIALS AND METHODS

The present experiment was conducted during the period from May to August 2008 at the experimental buffalo's farm of Animal Reproduction Research Institute (ARRI) Al-Ahram, Giza.

Experimental Buffaloes: This study was carried out on 10 multiparous dairy buffalo-cows (aged 3 to 7 years) during the 2nd to 5th parities. All animals were fed on balanced ration formulated to meet established nutrient requirements of pregnant and lactating dairy animals [24]. and water *ad-libitium*.

Ultrasonic Scanning: Buffaloes were submitted to a careful ultrasound examination weekly from day 8 post partum until first post partum ovulation to characterize the ovarian and genital tract changes [25]. A real-time, B-and M-mode linear array ultrasound scanner (480 vet. pie. Medical CO) which provided with trans-rectal transducer (5 and 7.5 MHZ) is used in this study.

Body Condition Score: Evaluations of body condition score were made in week 1 pre partum and in weeks 2 and 5 post partum on a scale of 1 to 5 (very thin to very fat) [26].

Milk Yield Milk: Yield was recorded at time of milking; between 5 and 7 AM and between 4:30 and 6:30 PM. It was carried at third and fifth weeks of lactation.

Blood Samples, Measurement and Analysis: Blood samples were taken from the jugular vein of all animals early in the morning, weekly from a week pre partum till 5 weeks post partum. Serum was separated by centrifugation and stored at -20°C until assayed for biochemical analyses except serum for the determination of Ac was stored at 4°C and was analyzed within 1 week after sampling. Serum concentration for ketone bodies (acetone + acetoacetate) was determined according to Pawan [27]. Serum concentration of βHBA was determined spectrophotometrically [28]. For acetone measurement, sample preparation was prepared according to Andre and Coppin [29] then analyzed using GC (model 5890 Hawellete Packared series II equipped with a flame ionization detector). Triglycerides were determined using specific kits (Spectrum Diagnostics; Egyptian Company for Biotechnology) [30]. Serum fatty acids were extracted with chloroform and methanol according to Loor *et. al.* [31] then methylated according to Karawya *et. al.* [32] and assayed using GC 5890 Hawellete Packared series II equipped with a flame ionization detector.

Statistical Analysis: Data were statistically analyzed [33] using ANOVA and represented as mean±SE.

RESULTS

Parameters for first post partum ovulation, body condition scores changes and milk yield are summarized in Table 1. Duration until first ovulation were longer ($P<0.05$) in LR than ER group. Body condition score (Bcs) decreased after parturition in both groups. Bcs changes were none significantly lower in LR than ER group while the milk yield was higher ($P < 0.05$) in LR than ER group Concentration of serum ketone bodies increased ($P < 0.05$) at the first week pre-partum as well as during 2nd, 4th and 5th weeks post partum in the LR compared with ER (Figure 1). Spectrophotometric analysis of βHBA in sera of buffalo cows showed increased ($P< 0.05$) in its concentration during the first week pre-partum as well as during first, fourth and fifth week post partum in LR than ER group (Figure 2).

The chromatographic panel of acetone concentration in sera of buffaloes showed increase ($P < 0.05$) acetone concentration during the 1st week pre-partum, day of calving, 1st, 2nd and 5th week post-partum in LR than ER group (Figure 3).

Measurement of serum triglycerides revealed increased ($P < 0.05$) concentration in ER than LR throughout the sampling period except at the first week pre-partum (Figure 4).

Table 1: Body condition score and milk yield of periparturient buffaloes in relation to resumption of post partum ovarian

	Classification of ovarian activity	
Item	ER	LR
Return to ovarian activity (d.p.p.)	26±3	45.8±0.70*
Bcs changes		
From weeks -1 to 2 of calving	0.16±0.20	0.22±0.18
From week 2 to 5 of calving	0.33±0.11	0.44±0.20
Milk yield till 5th week of calving	8.33±0.41	11.29±0.56

** $P < 0.05$. d.p.p. Days Post Partum

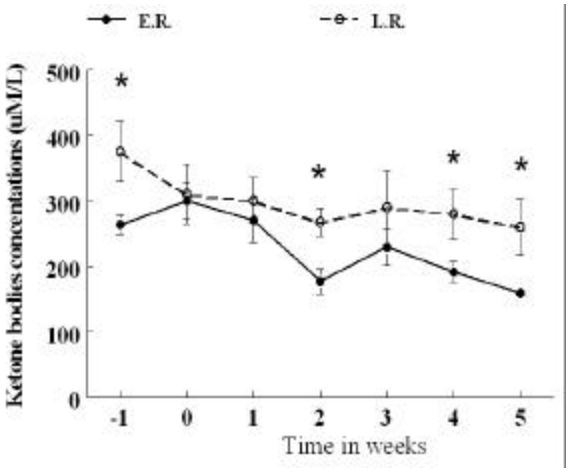

Fig. 1: Serum ketone bodies concentrations (µM/L) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

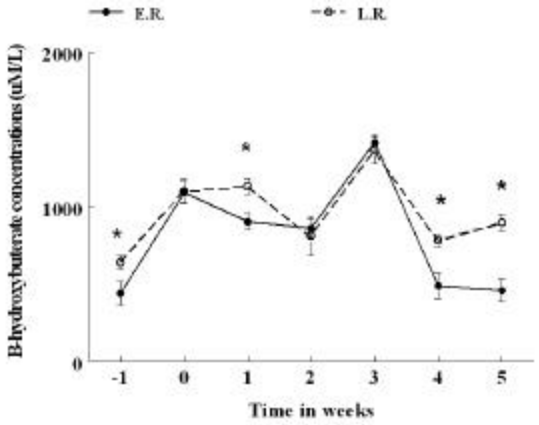

Fig. 2: Serum B-hydroxybuterate concentrations (µM/L) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

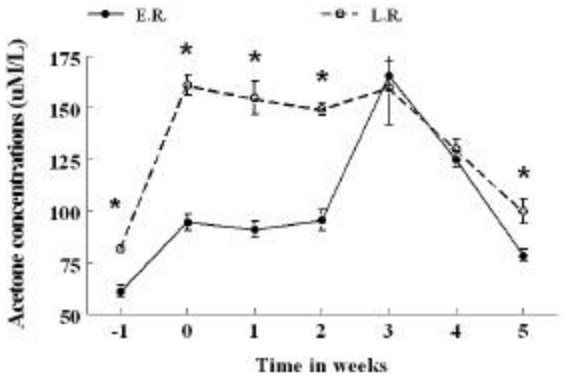

Fig. 3: Serum acetone concentrations (µM/L) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

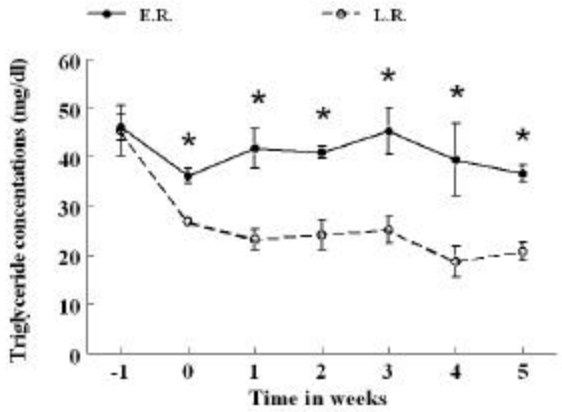

Fig. 4: Serum triglycerides concentrations (mg/dl) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

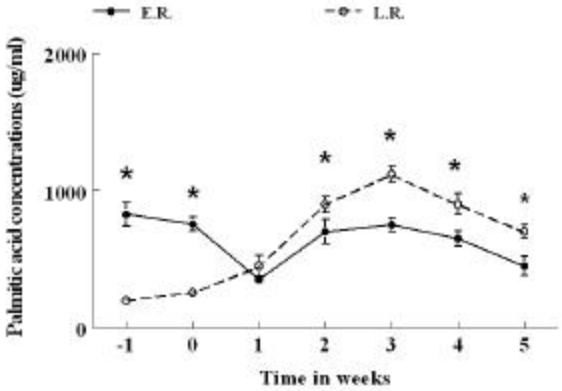

Fig. 5: Serum palmtic acid concentrations (µg/ml) in buffaloes differing in ovarian activity postpartum (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

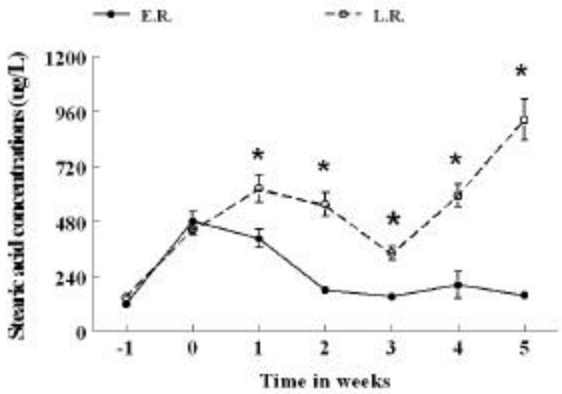

Fig. 6: Serum stearic acid concentrations (µg/ml) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

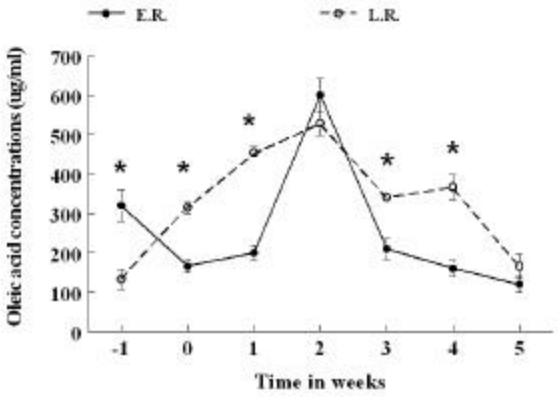

Fig. 7: Serum oleic acid concentrations (µg/ml) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

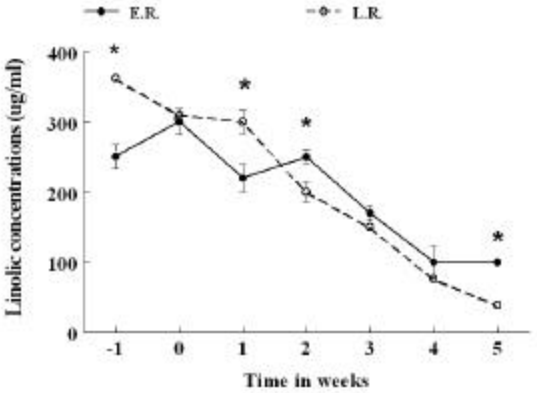

Fig. 8: Serum linoleic acid concentrations (µg/ml) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

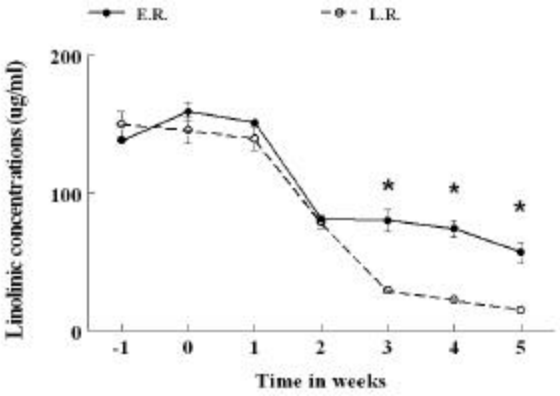

Fig. 9: Serum linolenic concentrations (µg/ml) in buffaloes in relation to resumption of post partum ovarian cyclicity (E.R. = early resumption to ovulation post partum, L.R. = late resumption to ovulation post partum). *P<0.05.

The concentration of serum palmtic acid (C16) was higher ($P < 0.05$) in ER at pre-partum and day of calving then decreased ($P < 0.05$) during 2nd tell 5th weeks post-partum compared with LR group (Figure 5).

The stearic acid concentration in sera of buffaloes (C18) was increased ($P < 0.05$) during post partum in the LR than ER group (Figure 6).

Serum oleic acid (C18:1) concentration was high ($P < 0.05$) during pre-partum in ER group. At calving and during the studied post-partum periods, it increased ($P < 0.05$) in LR group all over the whole period except at the 2nd and 5th weeks (Figure 7).

Serum concentration of linoleic acid (C18:2)was higher ($P < 0.05$) during the first week pre-partum and first week post-partum at the LR than ER group then decreased in both groups. Concentrations were higher ($P < 0.05$) at the second and fifth week post-partum in ER group than LR group (Figure 8)

Serum concentration of linolenic acid(C18:3) was higher ($P < 0.05$) during the 3rd, 4th and 5th weeks post-partum in the ER group than LR group.

DISCUSSION

In this study the measured concentrations of serum ketone bodies, triglycerides and fatty acids during the periparturient period in all buffaloes were in normal range. The multiparous buffaloes in this experiment were classified into (ER) and (LR) according to the examination results by ultrasonography The threshold value for classification of early responders has been set at 30 days This is considered optimal under practical condition in cattle [4]. The ER and LR buffaloes were in body condition score (2¼ minimum -2¾ maximum) at last 3 weeks of pregnancy. Nevertheless the loss of body condition during the first 5 weeks of lactation reflected utilization of body fat as a source of energy for milk production BCS losses were similar in both groups although this loss was not significantly greater in LR group. This reflected a trend for higher energy demands in LR than ER group. This is in agreement with Reist *et. al.* [4] and Ingevartsen and Anderson [34] in periparturient cows. Milk yield was determined for both groups until 5th week post partum. The milk yield in LR group was obviously higher than ER group. The current results showed clear increase of serum ketone bodies (acetone and acetoacetate) concentrations in LR group than ER at one week prep-partum, 2nd 4th and 5th week postpartum which indicated increase energy demands and oxidize large amounts of fatty acids in liver cells and consequently produce ketone bodies. Similar results were observed by Namara [35] in postpartum dairy cows. Measuring serum βHBA indicated transient elevation which was more marked and prolonged in LR group. This elevation was significant by a week before parturition and during 1, 4 and 5 weeks post parturition at LR than ER .These results indicated that metabolic stress was obvious in late ovarian group than early one also the significant elevation of ketone bodies might be responsible for the late return to ovarian activity [4, 16] which is in agreement with our results. Measuring serum acetone in both groups giving more information about this period The serum acetone concentration was significantly increased at 1st week pre-partum till the 5th week postpartum expect at 3rd and 4th week in LR group than ER group with peak at 3rd week postpartum. This result indicated enhanced ketogenesis as a consequence of enhanced fat mobilization and glucose shortage due to requirements of milk production with peak of lactation at the 3rd week. Similar results were previously observed Aeberherd *et al.* [36] who studied changes in metabolic traits in early lactation cows. Maximal values of ketone bodies seem to be most suitable to predict the onset of ovarian cyclicity and the greater the maximal concentration of ketone bodies in blood ,the greater the extent of incomplete oxidation of fatty acids before onset of estrous cycle the longer the interval from parturition to 1st ovulation lasted [4,16].

Triglycerides in serum showed higher level in ER than LR group during postpartum period .Several explanations were probably responsible for this results because the hepatic or intestinal origin of these triglycerides is unknown. One of these explanations was that buffalos in ER group were more efficient in the utilization and exporting of energy yielding substrates than LR buffaloes. Murondoti *et al.* [37] observed similar results in cows with fatty liver. In high producing cows fatty liver is a common postpartum condition [16, 38] that may be due to triglycerides accumulate within hepatocytes impairing their function. The other explanation for decreasing serum triglycerides in LR group was the portioning of stored energy substrates for milk synthesis [37].

Current results showed that concentrations of long chain fatty acids (Palmtic and oleic) decreased pre-partum then showed transiently increased after calving in ER group while, in LR group there were increased pre-partum and continued postpartum indicating early mobilization of lipids due to metabolic stress in LR group. But, stearic fatty acid concentration in serum of ER group decreased after calving suggesting that stearic acid is used by the liver (i.e. oxidation) or inconsiderably secreted through the milk [39].

The present results indicate more lipolysis in the LR group as previously shown [40] as indicated by rise in long chain fatty acids during lactation expressed enhanced adipose tissue mobilization to cope with high energy demand during that time. Recently, Douglas *et al.* [41] reported that the profiles of long chain fatty acids in serum were changed during the peripartum period in dairy cattle regardless of dry period dietary treatments as a result of adipose triglycerides mobilization that may explain our results. Both linoleic and linolenic fatty acids concentrations continuously decreased after parturition in the two experimental groups. There was significant increase of serum linoleic concentration at the 2nd and 5th week in ER group than LR group .Also linoleic acid increased at the 3rd, 4th and 5 th weeks in ER group .These changes in the diversion of linoleic and linolenic acids in both groups are unclear perhaps it may be related to restoration of body lipid pools. This explanation is consistent with Loor *et. al.* [42].The significant increase of linoleic and linolenic acids in ER group than in the LR group seems to have the most remarkable improvement effects on resumption of ovarian activity of buffalo .This finding is in consistent with Medoza *et. al.* [43] who said that essential fatty acids could modify follicular dynamics and reduce the length of the interval from calving to first ovulation .Santos *et. al.* [44] added that it's not completely clear whether these effects are mediated only by essential fatty acids or by other potential fatty acids during late gestation and early lactation.

It was concluded that significant elevation of ketone bodies concentration for longer time, may be reflected on the late onset of the ovarian cycle. Not only elevated ketone bodies but also increased long chain fatty acids concentration (palmtic, stearic and oleic) and diversion of linoleic and linolenic could explain the resumption of ovarian activity .The point must be stressed that this study was performed with healthy buffalos in which fat mobilization was not excessive (based on BCS, ketone bodies and fatty acids concentration). In true problem herds, application of ketone bodies measurements (especially serum acetone concentration) are expected to be of even greater practical importance.

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