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A FIELD TRIALS FOR MONITORING THE POST-PARTUM OVARIAN ACTIVITY IN DAIRY COWS

(With 5 Tables and 3 Figures)

By

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محاولات حقلية لمراقبة نشاط المبيض بعد الولادة فى الأبقار الحلوب

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أجريت هذه الدراسة على عدد ٤٥ بقرة حلوب تحت الظروف الحقلية حيث تم ملاحظة جميع الأبقار مرتين قبل الولادة وبعد الولادة لمدة ثلاث أشهر متتالية لملاحظة بداية نشاط المبيض وقد تم قياس دلالات حالة الجسم (Body condition score) وكذلك تم اخذ عينات الدم مع فحص كل الحالات تناسلياً وتم تسجيل الفحص التناسلى وكذلك تم التأكد من أول نشاط للمبيض عن طريق استخدام جهاز الموجات الفوق صوتية والجس المستقيمى. الأبقار التى بدأت أول نشاط للمبيض خلال ٣٠ يوم بعد الولادة صنفت على أنها ذات استجابة مبكرة (Early Responders) والأبقار التى أظهرت نشاط متأخر للمبيض صنفت على أنها ذات استجابة متأخرة (Late Responders) وقد تم قياس الأجسام الكيتونية (Ketone bodies) وهى عبارة عن بيتاهيدروكسى بيترات(BHBA) فى المصل ، أسيتات الأسيتون (ACAC) والأسيتون (AC) فى المصل واللبن وكان تركيز تلك الأجسام فى المصل واللبن خلال الستة أسابيع الأولى من إدرار اللبن مرتفعاً فى الحالات ذات الاستجابة المتأخرة عن الحالات التى أظهرت نشاط مبكر للمبيض بعد الولادة ولقد تم استنتاج أن قياس تركيزات الأجسام الكيتونية بعد الولادة مباشرة إلى ظهور أول نشاط للمبيض تعتبر أفضل دليل على بداية ظهور الشبق وقد وجد أن تركيز أسيتون اللبن وبيتاهيدروكسى بيترات المصل يعتبران من أفضل الأجسام الكيتونية التى تعبر عن نشاط المبيض بعد الولادة.

SUMMARY

The present study was carried out on a total number of 45 multiparous Holstien – Friesian dairy cows under field conditions. Cows were visited twice antipartum and daily postpartum for 3 successive months for observation of ovarian activity between 7: 30 and 9:30 at morning and between 6: 30 and 7: 30 in the evening. Body condition scores was measured and blood and milk samples were taken. All cows were

gynaecology examined and parameters of reproduction were determined. The onset of the first ovarian activity was specified by rectal palpation and by ultrasonograph. Cows starting postpartum ovarian activity within 30 days were considered early responders (ER) and cows starting postpartum ovarian activity later (more than 30 days) were considered late responders (LR). Ketone bodies measured were B-hydroxybutyrate in serum and acetoacetate and acetone in serum and milk. Blood serum and milk ketone body concentrations during the first 6 weeks of lactation were higher in LR than in ER. Ketone bodies from calving to first ovarian activity were better for prediction of the onset of estrus cycle. Milk acetone and serum B- hydroxy butyrate concentrations provided the most reliable information of all ketone bodies with regard to resumption of ovarian activity.

Key word: Ovarian activity, dairy cows.

INTRODUCTION

Attempts have been made to link the interval between calving and first ovarian activity to metabolic status. A significant, positive relationship has been observed (Allrich *et al*, 1987 and Butler *et al.*, 1981) between mean energy balance over the first weeks postpartum and the interval to first ovulation. In contrast, others (Villa- Godoy *et al.*, 1988 and Spicer *et al.*, 1990) were unable to relate mean negative energy balance with duration of postpartum anestrus. In the studies reported by Butler *et al.* (1981) and Canfield *et al.* (1990), first ovarian activity occurred approximately 10, 14 and 15 days after maximal negative energy balance, respectively. At ovulation mean energy balance was still negative, but in all cases was returning toward zero.

During early lactation the amount of energy required for maintenance of body tissue functions and milk production is well known to exceed the amount of energy that cows can obtain from dietary sources. Most glucose is directly used for milk synthesis, while glucose utilization for oxidation in extra mammary tissues is reduced (Baumann *et al.*, 1988). Therefore, cows must utilize body fat as an energy source. However, limited amounts of fatty acids can be oxidized to completion by the tricarboxylic acid cycle of the liver or be exported from the liver as very low density lipoproteins. In the case of excessive fat mobilization, associated with marked formation of acetyl- coenzyme A, the tricarboxylic acid cycle can not fully metabolize fatty acids (Baird, 1982). A consequence, acetyl- coenzyme A is converted to acetoacetate

(ACAC), which is then reduced to B- hydroxybutyrate (BHBA) by BHBA- dehydrogenase or spontaneously decarboxylized to acetone (AC) (Holtenius and Holtenius, 1996).A negative energy balance postpartum not only contributes to increased ketogenesis (Blum *et al*, 2000) but also delays the onset of ovarian cyclicity, especially if energy deficiency is prolonged (Anderson, 1988, Lucy *et al.*, 1991 and Zurek *et al.*, 1995).

One important mechanism by which energy deficit impairs reproductive activity is by suppressing the LH- releasing hormone (LHRH) and the pulse frequency necessary for ovarian follicle to grow to the preovulatory stage (Schillo, 1992). Mean plasma LH concentrations and number of episodic LH peaks increase after the maximal negative energy balance (Canfield and Buttler, 1991), and first ovulation occurs soon after for most cows.

Based on that, the present study was tested that differences in concentrations of blood and milk ketone bodies, plasma metabolites, and milk components during the first 6 weeks postpartum as contributed to differences in the onset of postpartum ovarian cyclicity in healthy dairy cows under field conditions. In addition, to determine whether concentrations of ketone bodies and other metabolites in blood and milk prior to first ovarian activity postpartum are most reliable with respect to prediction of the onset of ovarian cycle.

MATERIALS and METHODS

Animals:

Fourty five multiparous Holstein- Friesian dairy cows (aged 5 to 8 years) were included in the present study belonging to small farmers at Menofia and Qulyobia governorates. The herds of the studied farms were small (7 to 10 cows/ farm), and thus, the majority of cows calving in the period of investigation was included in the study. Cows were visited twice antipartum and daily postpartum for three months period between 7: 30 and 9:30 A.M and between 6: 30 an 7: 30 P.M. Time of sampling was based on previous studies on variation of ketone bodies (Blum *et al.*, 2000). The sampling period lasted from March 2002 until February 2003. In the studied cows there was no cases of clinical ketosis. Two groups were formed based on differences in onset of ovarian activity. Early responders (ER, n= 24 or 53.3% of cows) showed first observed heat within 30 days after parturition which is considered optimal under practical conditions (McClure, 1994). Late responders

(LR, n=21 or 46.71%) were cows with first observed heat between 35 and 87 days pastpartum.

Body condition Score

Evaluation of body condition scores (BCS) were made in the last two weeks antepartum and in the following six weeks postpartum according to Edmonson *et al* (1989).

Blood Analysis:

Twenty ml blood samples were taken from the jugular vein early in the morning, by use of evacuated tubes containing dipotassium-EDTA (1.8 g/L) or without anticoagulant in weeks 2 and 1 antepartum and in weeks 1,2,3,4,5 and 6 postpartum. Blood samples with the anticoagulant were put on ice, wherease tubes without anticoagulant (for recovery of serum) were left at room temperature until clotting. Tubes were then centrifugated for 20 minutes at 1500xg within 4h after collection. Serum for the determination of acetone (AC), acetoacetate (ACAC) and β- hydroxybutyrate (BHBA) and plasma for determination of glucose and non esterified fatty acid (NEFA) were stored at- 20°C until assayed according to Bruckmaier *et al* (1998). Serum for determination of AC and ACAC was stored at 5 C° and was analyzed within one week after sampling. Concentrations of AC and ACAC were determined as described by Marstop *et al.* (1983).

Milk samples:

Milk samples were taken in the morning for determination of protein, fat, lactose, ACAC and AC in weeks 1,2,3,4,5, and 6 postpartum according to the method described by Andersson (1984).

All the blood and milk parameters were determined at the central lab of the Faculty of Agriculture, Moshtohr, Benha (Zagazig Univ.).

Clinical observations of parturient cows:

Cows were submitted to a careful gynaecological examination in weeks 1,2,3,4,5 and 6 of lactation to characterize the status of genital tract and to detect possible health problems. Signs of estrus and intervals from calving to first abserved heat were determined by rectal palpation and by ultrasonography (Fig. 1, and 2). Interval from first estrus to first service and number of services perconception and conception at first service were also calculated. Early pregnancy diagnosis was determined by rectal palpation and confirmed by ultrasonograph (figure, 3).

Statistical Analysis:-

Data obtained was statistically analyzed using statistical Analysis System (SAS) (1987).

RESULTS

Clinical observations of parturient cows:

As shown in Table 1, Average age and number of parity and incidence of diseases incidental to parturition were much higher in LR than in ER. Duration until first observed heat and time until first service and number of services perconception were longer in LR than ER.

Body condition scores and blood traits:

As shown in Table 2, BCS decreased continuously after calving in both groups. BCS after parturition were always non significantly lower in LR than in ER.

Concentrations of glucose decreased abruptly after parturition, followed by slight increase in both groups and throughout the sampling period.

Antepartum BHBA was measurable in both groups. After calving BHBA concentration increased in LR but not in ER. In LR group it reached peak values at weeks 3 to 5. Concentrations of BHBA were higher in LR than in ER in weeks 3 and 5 postpartum.

Concentration of NEFA slightly increased Antepartum, increased markedly after calving then steadily decreased in both groups. Concentrations of NEFA were not significantly higher in LR than in ER throughout the sampling period.

Antepartum ACAC was below of detection in both groups. After calving ACAC concentrations increased in limits in both groups and in LR reached peak values in weeks 3 to 5. Concentrations of ACAC were higher in LR than in ER in week 3 and 5 post partum.

Antepartum AC was below detection in both groups. After calving AC concentrations increased in both groups and in LR reached peak values in weeks 3 to 5. It was higher in LR than ER at week 5 post partum.

Milk Traits:

As shown in Table 3. Concentrations of ACAC in milk were similar during first 2 weeks of lactation in both groups and LR reached peak levels from weeks 3 to 5. Concentration of AC in milk slightly increased during the first 2 weeks of lactation in ER and LR and LR,

reached peak levels from weeks 3 to 5. Concentrations of AC were higher in LR than in ER in weeks 3 and 5 postpartum. Milk protein concentration was higher in week 1, decreased rapidly until week 4, then remained at this level until week 6 in ER and LR. Concentrations of milk fat were highest in week 1, lowest in weeks 6 for both groups and slightly lower in ER than in LR throughout the whole period. There were no group differences in milk urea in both groups along the whole period and were non significantly higher in ER than in LR.

Correlations between ketone bodies:

As shown in table 4, all ketone bodies were closely and significantly correlated with each other. Correlation coefficient between serum BHBA and milk ACAC were lowest and coefficient between serum AC and milk AC were highest.

As shown in table 5, there were positive and significantly correlations of mean, maximum or minimum of BCS, blood and milk ketone bodies concentrations with Time until first postpartum observed heat.

DISCUSSION

Based on the time needed for parturient cows to return their ovarian cyclicity we classify cows into an early responders (ER) which return to first postpartum ovarian activity within 30 days and a late responders (LR) which need more than 30 days to regain their postpartum ovarian activities (McClure, 1994).

The average age was nearly equal in both groups and therefore, did not account for differences in ovarian activity. Incidence of diseases incidental to parturition were higher in LR than ER especially for retained placenta. The incidence of retained placenta is positively correlated with occurrence of ketosis (Burns *et al.*, 1997 and klerx and Smolders, 1997). However, the process of shedding of placenta starts before parturition, there was no obvious difference in Ketone bodies between group before parturition. It is important to note that effects on occurrence of first postpartum ovarian activity were not influenced by management factors, because of the equal distribution of cows under investigation within farms for both ER and LR. The time until first observed heat was much lower in ER (19 ± 1.32 days) than in late responder (62 ±4.31). Time until first service controlled by the farmers was 52 ±3.21 days for ER and 62 ±4.31 days for LR. Number of services

per conception and conception at first service were nearly similar in both groups. Anearly similar finding was observed by Zurek *et al* (1995).

Both ER and LR cows were in a fat body condition at parturition. However, the incidence of periparturient metabalic diseases was not observed as in other studies (Stärk *et al.*, 1997). Moreover, the loss of body condition during the first 6 weeks of lactation reflected utilization of body fat as a source of energy for milk production. BCS lossess were similar in both groups, although its loss in LR was non- significantly greater. This reflects slightly higher concentrations of milk fat, plasma NEFA and ketone bodies in LR than in ER.

The increase in concentration of NEFA from weeks 2 to 1 antepartum might have been due to a concomitant decreased feed intake (Grummer, 1993). The decrease of glucose concentrations of plasma after parturition could be expected (Baumann *et al.*, 1988). However, determinations of both glucose and NEFA levels did not allow us to draw a conclusions with regard to differences in energy metabolism between groups in this study. This is in agreement with Windisch *et al* (1991). They indicated that homeostatic control of glucose was largely maintained.

As a consequence of incromplete oxidation of fatty acids, postpartum concentrations of ketone bodies increased to prepartum values in both growth except for BHBA. On other hand BHBA is formed by reduction of ACAC and is synthesized by ruminal hydroxylation of butyrate, explaining why BHBA was measurable already before parturition (Zurek *et al.*, 1995). In contrast to ACAC and AC, BHBA can be partly used for the synthesis of milk fat. In ER, the amount of BHBA used for milk fat synthesis might have equaled the amount of BHBA formed by reduction of ACAC, explaining why BHBA did not significantly differ from preparturient values throughout the first 6 weeks of lactation in ER (Zurek *et al.*, 1995).

Ketone bodies in blood serum and milk in contrast to plasma glucose, plasma NEFA and milk components, exhibited clear differnces between groups. Concentrations of blood BHBA and AC, and milk AC were higher in LR than in ER during the first 6 week postpartum. This clearly indicates that incomplete oxidation of fatty acids, resulting from depletion of the tricarboxylic acid cycle, has been greater in LR than in ER. In agreement with results of a previous report (Playm -Forshell *et al.*, 1991), differences between groups and concentrations of ketone bodies were greater from weeks 3 to 5, although milk yield peaked from weeks 1 to 2 and typically maximum feed intake occurs 3 to 6 week later

than peak milk production. This is in accordance with Weaver (1987). Although available only in limited amountls, especially from labile proteins (Zurek *et al.*, 1995), use of free aminoacids for glucogenesis may have permitted the maintenance of a functioning tricarboxylic acid cycle in early lactation. Thus explaining why the concentrations of ketone bodies in the first two weeks postpartum were rather low based on ketone body determinations, depletion of tricarboxylic acid cycle, was marked in LR than in ER.

Correlations between the different ketone bodies determined in blood and milk were very strong as reported by several studies (Anderson, 1984 and Heyer, 1992).

From the present study, it could be concluded that, the onset of postpartum ovarian activity could be monitored by measurements of ketone body concentrations. It is well accepted that negative energy balance contributes to increased ketone body formation and delay the onset of ovarian cyclicity. Therefore we suppose that not only elevated ketone bodies, but also negative energy balances were more marked in LR than in ER. Milk AC is not only the most commonly used ketone body in herd monitoring programs for diagnosis of subclinical and clinical ketosis, but also turned to reflect the onset of ovarian cyclicity. Week 3 post partum is the best time for determination of milk AC in herd monitoring programs. Finally, application of ketone body measurements are expected to be of greater practical importance especially when the other updated facilites like hormonal assay and ultrasonography were out of question under field condition.

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Table, 1. Clinical observations of parturient cows under investigation

Item	Ovarian activity	
	ER (n = 24)	LR (n= 21)
- Return to ovarian activity (days post-partum)	≤30 (14-30)	>30 (35-87)
- Age (year)	5.5 ±0.20 [a]	5.6 ±0.21 [a]
- Parity	4.0 ± 0.03[b]	4.3 ± 0.03[a]
- Diseases incidental to parturition		
Dystocia	2	3
Retained placenta	1	4
Endometritis	1	1
-Reproductive activity (starting from calving until first observed heat (day)	19±1.32[b]	62±4.31[a]
Period until first service (day)	52± 3.11[b]	62±4.31[a]
Number of services per conception	1.36 ±0.16 [b]	1.50 ±0.14 [a]
Conception at first service (%)	64	53

Means with different superscripts are significantly different at level (P< 0.05)

ER= early responders cows (first observed heat within 30 days post –partum).

LR= late responders cows (first observed heat between 35-87 days post –partum).

Table 2. Body condition scores and blood traits in both early and late responders dairy cows (M ± S.E).

Items		Weeks Antepartum		Weeks post partum					
		-2	-1	1	2	3	4	5	6
BCS	ER	3.73 ± 0.1^{a}	3.71 ± 0.1^{a}	3.50 ± 0.1^{a}	3.3 ± 0.1^{b}	3.32 ± 0.1^{b}	3.32 ± 0.1^{b}	3.30 ± 0.1^{b}	3.30 ± 0.1^{b}
	LR	3.74 ± 0.1^{a}	3.72 ± 0.1^{a}	3.50 ± 0.1^{a}	3.31± 0.1^{b}	3.30 ± 0.1^{b}	3.25 ± 0.1^{bc}	3.09 ± 0.1^{bc}	3.00 ± 0.1^{c}
Glucose mmol/L	ER	3.20 ± 0.11^{a}	3.16 ± 0.10^{a}	2.20 ± 0.11^{bc}	1.90 ± 0.10^{d}	2.11 ± 0.10^{cd}	2.20 ± 0.1^{bc}	2.30 ± 0.1^{b}	2.30 ± 0.1^{bc}
	LR	3.20 ± 0.0^{a}	3.17 ± 0.10^{a}	2.39 ± 0.10^{b}	2.11 ± 0.11^{cd}	2.08 ± 0.10^{cd}	1.83 ± 0.1^{c}	2.40 ± 0.1^{d}	2.41 ± 0.1^{b}
BHBA µ mol/L	ER	580 ± 41.31^{d}	490 ± 17.31^{e}	583 ± 42.31^{d}	610 ± 40.31^{d}	598 ± 49.51^{d}	740 ± 38.97^{c}	631.3 ± 35.30^{d}	631 ± 35^{d}
	LR	581 ± 12.40^{d}	47 ± 16.53^{e}	711 ± 25.21^{c}	730 ± 41.30^{c}	1010 ± 89.30^{b}	997 ± 65.30^{b}	1366 ± 137.00^{a}	720 ± 34^{c}
NEFA µ mol/L	ER	120 ± 7.31^{g}	145 ± 12.4^{fg}	421 ± 28.31^{a}	310 ± 28.3^{b}	270 ± 11.31^{c}	271 ± 12.33^{d}	260 ± 11.40^{cd}	234 ± 18.31^{de}
	LR	123 ± 8.91^{fg}	151 ± 13.31^{f}	487 ± 40.31^{a}	397 ± 40.0^{ab}	330 ± 12.50^{b}	290 ± 14.33^{c}	271 ± 11.33^{cd}	198 ± 14.41^{e}
ACAC µ mol/L	ER	-	-	12 ± 4.30^{d}	43 ± 6.40^{c}	51 ± 7.37^{c}	104 ± 21^{b}	46 ± 5.31^{c}	42 ± 4.30^{c}
	LR	-	-	16 ± 5.37^{d}	56 ± 7.31^{c}	140 ± 14.30^{b}	241 ± 52^{a}	50 ± 8.1^{c}	59 ± 7.41^{c}
AC µ mol/L	ER	-	-	19 ± 3.44^{e}	51 ± 7.37^{d}	56 ± 8.441^{d}	120 ± 18.34^{c}	48 ± 3.4^{d}	46 ± 3.61^{d}
	LR	-	-	22 ± 5.31^{a}	82 ± 11.66^{c}	186 ± 14.30^{b}	192 ± 15.33^{b}	303 ± 8.4^{a}	101 ± 10.40^{c}

Means with different superscripts are significantly different at level (P< 0.05)

ER= early responders **LR**= late responders

BCS= body condition scores. **BHBA**= B- hydroxy butyrate.

NEFA= Non esterified fatty acids **ACAC**= actetoacetate

AC= acetone

Table 3. Milk traits in both early and late responders dairy cows along 6 weeks postpartum(M ± S.E).

Items		Weeks post partum					
		Ist	2nd	3rd	4th	5th	6th
ACAC µ mol/L	ER	10.90 ± 0.13^{d}	10.87 ± 0.11^{d}	3 ± 0.14^{e}	22 ± 3.91^{b}	14.3 ± 4.11^{bc}	10.98 ± 2.11^{cd}
	LR	3 ± 0.13^{e}	16.71 ± 2.66^{bc}	24 ± 3.73^{b}	29 ± 8.77^{ab}	48 ± 9.31^{a}	12.73 ± 2.34^{cd}
AC µ mol/L	ER	43 ± 7.90^{c}	47 ± 8.31^{c}	50 ± 9.47^{c}	85 ± 19.71^{bc}	52 ± 8.6^{c}	49 ± 6.33^{c}
	LR	108 ± 23.86^{b}	109 ± 20.77^{b}	210 ± 23.60^{a}	260 ± 17.30^{a}	248 ± 49.3^{a}	97 ± 8.93^{b}
Protein G/L	ER	39 ± 1.73^{a}	35 ± 1.77^{abc}	34 ± 1.66^{bc}	34 ± 1.34^{bc}	31 ± 1.30^{cd}	33 ± 0.4^{bc}
	LR	39 ± 1.43^{a}	36 ± 1.39^{ab}	34 ± 1.73^{bc}	34 ± 1.44^{bc}	24 ± 0.4^{e}	30 ± 0.4^{d}
Fat G/L	ER	48 ± 1.34^{b}	39 ± 1.36^{e}	40 ± 1.66^{e}	39 ± 1.44^{e}	39 ± 1.4^{e}	38 ± 1.33^{e}
	LR	52 ± 1.33^{b}	45 ± 1.77^{cd}	43 ± 1.66^{de}	97 ± 1.66^{a}	48 ± 1.33^{c}	36 ± 1.36^{e}
Urea	ER	38 ± 2.34^{cd}	39 ± 2.34^{b}	55 ± 3.45^{a}	44 ± 2.44^{bc}	43 ± 1.00^{b}	40 ± 1.78^{cd}
	LR	43 ± 1.31^{bc}	58 ± 2.93^{a}	28 ± 1.78^{e}	36 ± 3.4^{de}	32 ± 2.3^{e}	30 ± 2.31^{e}

Means with different superscripts are significantly different at levels (P<0.05).

ER= early responders LR= late responders

ACAC= acetoactate AC= acetone

Table 4. Correlations between ketone bodies in blood plasma, blood serum and milk.

	Milk		Blood plasma		
	AC	ACAC	AC	BHBA	ACAC
Blood serum					
ACAC	0.80*	0.73*	0.80*	0.83*	1.00*
BHBA	0.82*	0.70*	0.82*	1.00*	
AC	0.93*	0.77*	1.00		
Milk					
ACAC	0.80*	1.00*			
AC	1.00*				

Correlation coefficients are statistically significant (P<0.05).
AC= acetone. ACAC= acetoacetate. BHBA= B- hydroxybutyrate.

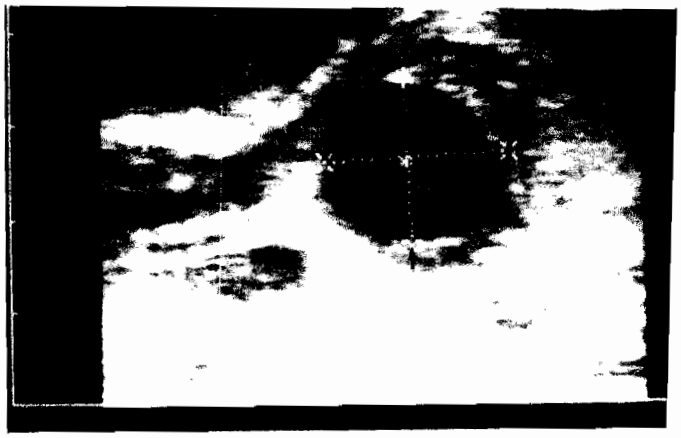
Table 5. Correlations of interval from calving until first observed heat with body condition scores, blood and milk traits.

Traits	Interval from calving until first observed heat		
	Mean	Maximum	Minimum
Body condition score	-0.24*	-0.06	-0.32*
Blood Traits			
NEFA	0.01	0.18	-0.12
Glucose	0.01	0.20	0.21
ACAC	0.29*	0.33*	0.02
BHBA	0.33*	0.42*	0.12
AC	0.23*	0.32*	0.02
Milk Traits			
ACAC	0.27*	0.42*	-0.03
AC	0.30*	0.38*	0.09
Fat	0.00	0.20	-0.24*
Protein	-0.44*	0.09	-0.52*
Urea	-0.09	0.14	-0.31*

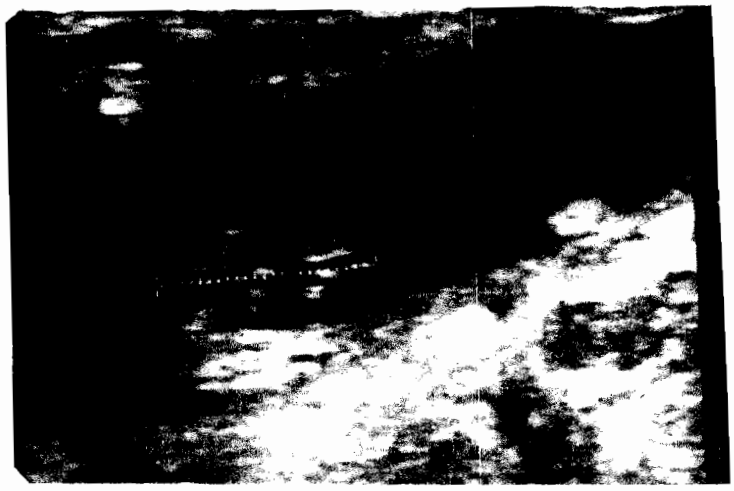
Correlation coefficients are significantly at (P<0.05).
AC= acetone. ACAC= acetoacetate. BHBA= B- hydroxybutyrate.
NEFA= Non esterified fatty acids

Zurek, E.; Foxcroft, G.R. and Kennelly, J.J.(1995): Metabolic Status and interval to first ovulation in postpartum dairy Cows J. Dairy Sci. 78: 1909-20.

Figure, 1: Ultrasonographic image of growing follicles with anechoic antrum and a very thin ill identified hyperechoic follicular wall, the diameter of small follicle, (2.40 mm) and for the medium one (3.33 mm) in size (arrow).



Figure, 2: Ultrasonographic image of mature graffian follicle with a size 14.36 mm (arrow).



Figure, 3: Ultrasonographic image of gravid uterus at 38 days. Note the embryonic mass at the apex of the gravid horn with CVRL= 1.73 cm (arrow).