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BIOCHEMICAL ANALYSIS OF SOME BLOOD CONSTITUENTS IN FEMALE BUFFALOES DURING LATE PREGNANCY AND PUERPERIUM

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ABSTRACT

The present study was designed to estimate the changes of some biochemical constituents in the blood of apparently healthy pregnant buffaloes during late pregnancy, parturition, early puerperium as well as during estrous and the diestrous phases. A total number of 137 blood plasma samples collected from 16 female buffaloes,5 control (non pregnant) and II pregnant at late stage were investigated for biochemical analysis of total proteins, creatinine, total lipids, phospholipids, total cholesterol and inorganic phosphorus.

The obtained data showed a siginficant decrease of plasma total proteins during the late month of pregnancy, followed by an increase postpartum and throughout puerperium. The data also revealed a significant decrease of plasma creatinine level throughout the late months of pregnancy and the first month postpartun. Plasma total lipids revealed a significant decrease during the late month of pregnancy, followed by an increase after calving. A significant decrease was recorded for plasma phospholipids during late month of pregnancy. The phospholipids level showed a significant increase during the first month and at the fourth week postpartun, followed by a significant increase during oestrus. The plasma total cholesterol level decreased significantly throughout the late month of pregnancy. A significant increase of total cholesterol level was observed at the fourth week post-partum and at oestrus. Plasma inorganic phosphorus increased significantly during late month of pregnancy.

INTRODUCTION

Pregnancy imposes a large demand upon the female animal. Pregnancy and parturition are physiological conditions which may be accompanied with some alterations in the biochemical constituents of the blood (Soliman et al., 1966).

Since the blood acts as the carrier for nutrients from the pregnant animal to its developing foetus, analysis of blood may reflect the nutritional status for the foetus. It also throws some light upon the food requirements of the dam during this critical

period of reproduction (El-Naggar, 1975).

Pregnancy imposed a large demand upon mother minerals, and afrer parturtion for the formation of colostrum, and the beginning of lactation which consituted heavy load upon mother minerals (El-Hariri et al., 1986).

David and Vincont, (1983). reported that, pregnancy and oestrogen contraception produced changes in the plasma concentration of trace elements in concomitant with the alteration in circulating plasma proteins.

The aim of the present study is to follow up the alteration of total proteins, creatinine, total lipids, phospholipids, total cholesterol and inorganic phosphorus in the blood plasma of pregnant buffalo-cows, throughout the last month of prrgnancy, early pureperium as well as at parturition. Hence the reproductive potential of buffalo-cows can be assessed.

MATERIAL AND METHODS

The present study was carried out on a total number of 11 sexually mature and pregnant buffaloes and 5 control (non pregnant) kept at the experimental farm, belonging to the faculty of Agriculture Science (Moshtohor), Zagazig University (Benha branch). They were clinically normal and proved to be free from the infectious and coital diseases as well as external and internal parasites.

Approximately ten mls of blood were collected from the jugular vein on EDTA as anticoagulant, every week during the last month of pregnancy, three successive days from the onset of parturition, then every week throughout the first month of the postpartum period and at the day of the first postpartum heat. Blood samples were also taken from another five non pregnant buffalo-cows clinically proved to be in the dioestrus or luteal phase, used as control group . Blood plasma was separated and kept at -2°C till the biochemical analysis performed.

Plasma total proteins, creatinine, total lipids and total cholesterol were determined colorimetrically by using kits-produced by. Egyptian American Co. for laboratory services ' according to the technique adopted by Gornall et al. (1949). Schimester (1964), Zollner and Kirsch (1962) and Ratliff and Hall (1973), respectively. Whereas plasma phospholipids were determined according to Zilversmit and Davis(1956) and plasma inorganic phosphorus was determined according to Drekh and Jung (1970).

The data obtained were statistically analysed according to the methods described by Denenberg (1976).

RESULTS

The results of the present study (Table 1), showed that, there was a significant decrease of plasma total proteins during the late month of pregnancy, followed by an increase at postpartum and throughout puerperium. Table (1) also showed a significant decrease of plasma creatinine level throughout the first three days postpartum, but it was significant throughout the first month postpartum.

Plasma total lipids showed a significant decrease during the late month of pregnancy, followed by an increase after calving.

Plasma phospholipids revealed a significant decrease during the late month of pregnancy but not during the first three days postpartum. There was a increase in

plasma phospholipids during the first month postpartum bu it was significant at the fourth week in postpartum. Asignificant increase of plasma phospholipids level was recorded at oestrus (Table 2).

A significant decrease of plasma total cholesterol level throughout the late month of pregnancy. There was a non significant increase in plasma cholesterol level during the first three days of the postpartum period which became significant at the fourth week. There was a significant increase in the level of cholesterol at oestrus. (table 2).

Plasma inorganic phosphorus increased significantly during the late month of pregnancy and then decreased (but not significantly), throughout the first three days in postpartum. There was a non significant increase in the level of inorganic phosphorus during the first month of postpartum. A non significant variation in plasma total proteins and total lipids level between estrous and the dioestrus phases was observed whereas, there was a significant variation in the level of inorganic phosphorus between oestrus and dioestrus phases (Table 2).

DISCUSSION

During pregnancy, it has been emphasized that the plasma total proteins decline as the animal approaches time of birth (Larson and Kendall, 1957, Schalm, 1975; El-Baghdady, 1984; Abdel-Maksoud, 1991) . This finding came in consistent with that shown in the present study where the total proteins steadily decreased from the fourth to the first week before parturition (Table 1) . Such decrease in the plasma total proteins was attributed to the great exchange in globulins from blood to mammary glands as colostrum (Larson and kendall, 1957 and More et al., 1971) and to the rapid progress in the uterus and its contents (Mourad et al., 1983).

Immediately and throughout the first three days after parturition, the present study revealed a non significant increase in the plasma total proteins , a finding which came in accordance with some previous studies (El-Baghdady, 1984) . The increase in the plasma total proteins level although it was non significant might be attributed to increasing the feeding intake which possibly surpasses the maintenance and productive capacity of the animal.

Following the first three days afrer parturition, the present study monitorized a significant prgressive increase in the plasma total proteins from the first to the fourth week postpartum. This postparturient increase in the plasma total proteins, was observed in buffaloes (El-Naggar, 1975), cows (Larson et al., 1980) and the camels (Shehata, 1987). The significant increase in the plasma total proteins could be attributed to increasing the globulins in blood as a squellae of invading the uterus by mild infection after parturition without clinical signs of mucometra or pyometra, but with prolongation in the clinical uterine involution (Roberts, 1971).

Parallel to the decline in the total proteins, the plasma creatinine,which is the catabolic product of the total nitrogenous compounds (Bath et al., 1985), appeared to decrease remarkably from the fourth to the first week before parturition. The present value came nearly in consistent within the range reported earlierly (Salah et al., 1976). Since creatinine has a positive relationship to protein metabolism (Marshal and

Hughes, 1980), thus increasing plasma creatinine is used as indication to excessive protein metabolism and vice versa. This finding could be anticipated in association with the progress in pregnancy (Salem, 1980, Kaldas and Henin, 1992). However, the decline in plasma creatinine, can be explained in two ways. First, the decrease in plasma total proteins is mainly anabolic and not catabolic due to the great exchange in globulin from blood into the mammary, glands as colostrum.Second, the protein, metabolism in its anabolism runs at low steady rate during the last month of pregnancy where the foetal growth is completed and the body metabolism is maintained.

After parturition and throughout the first month of postpartum, the plasma creatinine still showed a decline in its level contrary to that observed in case of the plasma total proteins. This finding might support the positive relationshipbetween plasma creatinine and protein metabolism (Salem, 1980, Kaldas and Henin. 1992).

The pesent study showed, a decrease in level of the total lipids during late pregnancy, such decrease during late pregnancy came in accordance with that shown in some reports (Dunkan and Garton, 1963,prakash and Tandon,1979,Raphael et al., 1973 and Eissa, 1988). The decline in total lipids is related mainly to the gradual exchange of fatty acids containing lipids from the blood into the mammary glands . The lowest level of plasma total lipids appeared at the zero day of parturition after which they began to increase, a finding which came in support with some previous studies (Raphael et al., 1973).

Throughout the first month of puerperium the present study indicated an increase in the plasma total lipids, a finding came in consistent with some of previous studies (Avidar et al., 1981 and prakash and Tandon, 1979).

The present study revealed a significant decline in level of cholesterol throughout the last month of pregnancy, a finding which came in accordance with (Shehata, 1987, Salem, 1980 and Rowlands et al.,1980). The decline in level of total cholesterol throughout the last month of pregnancy might came in association with the gradual increase in level of oestrogen as a predisposing factor initiating the mechanism of parturition (Hafez, 1986). It has been reported that oestrogen has significant relationship to the plasma total cholesterol (Glomset and Williams,1963, Purohit and Kohli, 1977).

At parturition the present study showed a remarkable decline in level of plasma cholesterol on the zero day of parturition followed by a non appreciable increase throughout the first three days after parturition. This finding came in agreement with that mentioned in some previous reports (Dunkan and Garton, 1963; Jadhav et al., 1971 and Sohukar et al., 1985) .

Throughout the first month of the postpartum period there is a prominent increase in the level of blood cholesterol, a finding which came in consistent with some previous investigations (Avidor et al.,1981 ; Shehata, 1987 and Eissa, 1988), it has been indicated that cholesterol increased during early puerperium as the level of circulating and thyroid hormones decreased (Prakash and Tandon, 1979).

The present study indicated that the cholesterol level was significantly lower during the oestus phase than during the dioestrus phase, (Table 2) . This finding came in

consistent to that reported by Hassan et al. (1980) where cholesterol is higher in the luteal than the follicular phase, but differs from others (Purohit and Kohli, 1977) where cholesterol is high during the follicular phase. Thus the cholesterol may be lowered due to the effect of ostrogen during oestrus and may be higher by the effect of progesterone during dioestrus.

In the present study, a highly significant decline was observed in phospholipids during the last stage of pregnancy, a finding which came in agreement with that reported by Holtenius et al. (1986) and Eissa (1988). Immediately after and throughout the first three days, of parturition the level of phospholipids is still at its lowest particularly at the third day of puerperium.The remarkable decrease in phospholipids might be attributed to the great exchange of phospholipids into the mammary glands to be utilized in milk fat synthesis (Yang et al., 1978). On the other hand the level of phospholipids in blood begins to increase following the third day after parturition, a finding which came in consistent with. Holtenius et al., (1986), who indicated that level of phospholipids appeared much lower immediately after calving followed by an upward increase. However, Eissa (1988) noticed an increase in the level of phospholipids in buffaloes and cows following the third week of puerperium.

Nevertheless, in the current study the level of phospholipids appeared significantly higher in blood of buffalo-cows during the dioestrus phase when compared to those during the estrous phase (table 2) this findings suggested that the level of phospholipids might have a significant relationship to the effect of progesterone during dioestrus rather than the effect of oestrogen during oestrus.

The plasma inorganic phosphorus increased in its level particularly during the last week of pregnancy. The increased level of inorganic phosphorus with progress of pregnancy is usually assocaited with the great demand for phosphorus either in foetus development or in the carbohydrate metabolism especially during late pregnancy (Best and Talyer, 1950 and Ismail and Hussein, 1988), whereas, a significant decrease in the plasma inorganic phosphorus was observed at the end of the third trimester (Mohamed, 1989). However, the plasma inorganic phosphorus did not differ, between the different stage of pregnancy (Shehata and Zaghloul, 1990). Such difference in plasma inorganic phosphorus estimates is not paramont as the phosphorus supplied in the diet is enough to meat the requirements at pregnancy due to the involvement of plasma inorganic phosphorus in the active transport mechanism across the placenta (Wafaa et al., 1991).

Immediately and throughout the first three days after parturition, there is a decline in the plasma inorganic phosphorus, such fall in the phosphorus level may be related to increasing its utilization with the enhanced carbohydrate metabolism and its flow to the udder (Pathak and Jankiraman, 1987).

Throughout the first month of pureperium, the present study revealed a non significant increase in the plasma inorganic phosphorus, a finding which might be related to increasing the feeding intake of phosphours particularly for lactating buffalo-cows. However, the plasma inorganic phosphorus is significantly higher during the oestrus than that during the dioestrus. A finding which came in accordance with some

Table (1): Blood plasma constituents of buffalo-cows during the last month of pregnancy, parturition and postpartum period

Parameters / Statements	Mean ± S.E. gm/dl	Mean ± S.E. mg/dl				
	Total proteins	Creatinine	Total lipids	Phospholipids	Total cholesterol	Inorganic phosphorus
4th week before parturition	10.15 ± 2.08 a	2.11 ± 0.07a	427.27 ± 14.08a	98.60 ± 3.99a	144.11 ± 5.86a	5.32 + 0.19a
3rd week before parturition	9.91 ± 0.25a	2.00 ± 0.07a	368.18 ± 12.20b	82.27 ± 2.81b	136.98 ± 6.05a	5.26 + 0.18a
2nd week before parturition	8.91 ± 2.28b	1.89 ± 0.04b	322.73 ± 15.61c	69.10 ± 3.29c	117.46 ± 5.56b	5.65 + 0.17a
1st week before parturition	8.91 ± 0.27b	1.82 ± 0.04b	286.36 ± 17.95c	68.18 ± 2.63c	89.68 ± 2.60c	6.17 + 0.14b
Day of parturition	9.84 ± 0.27a	1.66 ± 0.05a	181.82 ± 12.70a	50.00 ± 3.02a	82.30 ± 2.82a	5.65 + 0.13a
One day postpartum	10.07 ± 0.34a	1.63 ± 0.06a	200.00 ± 16.51a	46.36 ± 3.02a	83.39 ± 2.02a	5.54 + 0.11a
Two days postpartum	10.09 ± 0.26a	1.63 ± 0.12a	218.18 ± 15.48a	47.27 ± 2.97a	84.57 ± 2.84a	5.48 + 0.10a
1st week after parturition	10.04 ± 0.31a	1.68 ± 0.06a	231.82 ± 10.16a	69.54 ± 4.44a	100.97 ± 5.40a	5.57 + 0.10a
2nd week after parturition	11.21 ± 0.32b	1.68 ± 0.06a	240.91 ± 16.26a	73.18 ± 3.89a	105.94 ± 4.59a	5.64 + 0.11a
3rd week after parturition	11.48 ± 0.14b	1.50 ± 0.08b	250.00 ± 16.51a	85.45 ± 5.45b	156.84 ± 9.10b	5.67 + 0.11a
4th week after parturition	11.30 ± 0.18b	1.45 ± 0.06b	304.54 ± 21.80b	90.45± 3.90b	166.29 ± 6.76b	5.68 + 0.12a

Data are presented as mean ± S.E.
a-b: Significant difference at (P<0.05)
a: Non significant difference at (P<0.05).
a-b-c: Significant at (P<0.05)

a,b,c, ... is the arrangement of significant value to the less one
Means with the same letter are not significantly different (P<0.05).

Table (2) Biochemical parameters in the blood of Buffalo-cows during estrous and diestrous phase.

Parameters / Statements	Mean ± S.E. gm/dl	Means ± S.E. mg/dl				
	Total proteins	Creatinine	Total lipids	Phospholipids	Total cholesterol	Inorganic phosphorus
Estrous phase	10.20 ± 0.23a	1.82 ± 0.07a	295.45±14.23a	41.82 ± 2.05a	77.57 ± 5.04a	7.17 ± 0.07a
Diestrous phase	10.25 ± 0.23b	1.77 ± 0.06 a	300.00±15.18a	67.00± 3.74b	109.32 ± 5.76b	6.60 ± 0.19a

a : Non significant difference at (P< 0.05). a-b : Significant difference at (P < 0.05).
a, b, ... is the arrangement of significant value to less one . Means with the same letter are not significantly different (P< 0.05)

previous studies (Gamy, 1991; El-Shawaf, 1984; Dhoble and Gupta, 1986 and Sherlata and Zaghloul, 1990). Such difference was attributed mainly to increasing the metabolic activity of oestrogen during the follicular phase (Osman et al., 1979). From the present study it can be concluded that in order to attain normal parturition, dropping of the fetal membranes at its actual time, enhancement of the uterine involution and shortening the interval from calving to the first observed heat, attention should be paid to increase the rate of some elements in the diet especially the total proteins, total lipids, phospholipids and inorganic phosphorus.

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التحليل البيوكيميائى لبعض مكونات الدم فى إناث الجاموس أثناء فترة نهاية الحمل والنفاس

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الغرض من إجراء هذه الدراسة هو قياس بعض المركبات الكيميائية الحيوية مثل البروتين الكلى، الكريانينين الدهون الكليه الدهون الفسفورية الكلسترول الكلى وكذلك الفوسفور الغير عضوى فى بلازما دم إناث الجاموس عند أهم المراحل الفسيولوجية التى تمر بها وهى فترة الحمل المتأخر والنفاس .

ولذا أجريت هذه الدراسة على ١١ من إناث الجاموس أثناء الشهر الأخير من الحمل وبعد الولادة بشهر وكذا خلال طور الشق فى مزرعة كلية الزراعة بمشتهر جامعة الزقازيق فرع بنها .

وقد تم الحصول على ١٣٢ عينة من الإناث الحوامل خلال الشهر الأخير من الحمل والثلاثة أيام الأولى بعد الولاده ثم شهر بعد الثلاثة أيام الأولى من الولادة وكذلك خلا ل طور الشق بالإضافة إلى خمسة عينات من الإناث قبل الحمل أستخدمت كعينات ضابطة . وقد تم فصل جميع عينات بلازما الدم وتم قياس بعض المكونات الأساسية بإستخدام جهاز الإسكترو فوتوميتر . وقد أسفرت النتائج بعد دراستها إحصائيا على ما يلى : لوحظ وجود نقص معنوى فى مستوى البروتين الكلى خلال الشهر الأخير من الحمل ثم إزداد خلال الشهر الأول بعد الولاده وكانت الزيادة معنوية فى الإسبوع الثانى بعد الولادة وأظهرت النتائج أيضا وجود نقص معنوى فى مستوى الكريانينين خلال الشهر الأخير من الحمل وكذلك خلال الشهر الأول بعد الولادة. لوحظ وجود نقص معنوى فى مستوى الدهون الكلية خلال الشهر الأخير من الحمل كما لوحظ وجود زيادة معنوية فى مستوى الدهون الكلية فى الأسبوع الرابع بعد الولادة . أما بالنسبة للدهون الفسفورية كان هناك نقص معنوى خلال الشهر الأخير من الحمل ووجود زيادة معنوية فى الأسبوع الرابع بعد الولادة وقد أظهرت نتائج الكلسترول الكلى على وجود نقص معنوى خلال الشهر الأخير من الحمل ووجود زيادة معنويه فى مستوى الكلسترول الكلى خلال الأسبوع الرابع بعد الولادة وقد أظهرت النتائج أيضا عن وجود زيادة معنوية فى مستوى الفوسفور الغير عضوى خلال الشهر الأخير من الحمل . لم يلاحظ أى إختلافات معنويه فى مستوى البروتين الكلى ، الكريانينين ، الدهون الكلية والفوسفور الغير عضوى بين الحيوانات التى فى طور الشق وتلك غير الحوامل أما بالنسبه للدهون الفسفوريه والكلسترول الكلى كان هناك زيادة معنويه فى العينات الضابطه عنها فى طور الشق .

ومن نتائج هذه الدراسة نستطيع ان نستخلص ما يلى: لكى نحصل على ولادة طبيعيه ونزول الأغشية الجنينية فى وقتها الطبيعى بعد الولاده ورجوع الرحم أيضا إلى حالته الطبيعيه وتقليل الفتره من الولاده إلى ظهور أول دورة شق ننصح الإهتمام بمكونات العليقه خاصه زياده معدل إضافه بعض العناصر مثل البروتين الكلى والدهون الكلية والفسفوليبيدات وأيضا الفوسفور الغير عضوى فى هذه الفتره الحرجة من الحمل والنفاس لما لها دور كبير فى إكتمال هذه المراحل الفسيولوجية.