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STUDY OF SOME BLOOD SERUM CONSTITUENTS DURING ESTRUS CYCLE AND ANESTRUS PERIOD IN EGYPTIAN BUFFALOES

(With 2 Tables)

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دراسة بعض مكونات مصل الدم خلال دورة الشياع وفترة الخمول الجنسى في الجاموس المصرى

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اجريت هذه الدراسة في محطة بحوث الانتاج الحيواني بمسطرد التابعة لقسم الانتاج الحيواني التابع اكلية الراعة جامعة الأزهر بالقاهرة. استخدم في هذه الدراسة عدد اربعة عشر جاموسة سبعة جاموسات اظهرت الشياع المصاحب بالتبويض وتم معرفة ذلك عن طريق الجس المستقيمي والسبع جاموسات الاخريات ظلت في فترة هدوء جنسي لاكثر من ٩٠ يوم بعد الولادة. واستهدفت الدراسة متابعة التغيرات في بعض مكونات مصل الدم والتي اشتملت على البروتين الكلى ، والالبيومين ، والجلوبيولين ، انزيم الفوسفاتيز القاعدي والانزيمات الناقلة لمجموعة الامين والكولسترول. اظهرت النتائج ان كلا من البروتين الكلى والالبيومين والجلوبيولين والكولسترول كانت عالية في يوم الشياع مقارنة بباقي ايام دورة الشياع وفترة الخمول الجنسي وكانت هذه الاختلافات معنوية. بينما النسبة بين الالبيومين والجلوبيولين كانت متذبذبة طوال دورة الشياع وكانت هذه النسبة في فترة اللاشياع منخفضة ومتشابهة مع السيوم الثامن والعاشر من دورة الشياع مقارنة بباقي ايام دورة الشياع الاخرى. بينما انزيم الفوسفاتيز القاعدى اظهر انخفاضاً عن يوم الشياع مقارنة بباقى ايام دورة الشياع وفترة الخمول الجنسي وكانت هذه الاختلافات معنوية. اما بالنسبة للانزيمات الناقلة لمجموعة الامين لوحظ أن انزيم الاسبارتيات امينوترانس فيرز كان عالى في يوم الشياع مقارنة بباقي ايام دورة الشياع وفترة الخمول الجنسي لكن كانت هذه الاختلافات غير معنوية. وبالنسبة لانزيم الالاليين امينوترانس فيرز فقد لوحظ ارتفاع نسبته بالدم عند نهاية دورة الشياع مقارنة بباقى ايام دورة الشياع وفترة الخمول الجنسى وكانت الاختلافات معنوية.

SUMMARY

Total proteins, albumin, globulin, A/G ratio, alkaline phosohatase (AP), aspartate aminotransferase (AST), alanine aminotronsferase (ALT) and cholesterol were studied in fourteen buffaloes, seven of them during

estrus cycle and the other buffaloes remained anestrus for 90 days or more postpartum. Total proteins, albumin, globulin, and cholesterol levels were high at estrus comparsion with other days of estrus cycle and anestrus period, these differences were significantly (P<0.05). A/G ratio was fluctuated throughout of estrus cycle. Value of A/G ratio in anestrus period was low and similar to 8 and 10 days of estrus cycle comparison with other days of estrus cycle. Alkaline phosohalase activity was low at estrus in comparison with anestrus period and other days of estrus cycle, these differences were significantly (P<0.05). Concentration of AST was high at estrus in comparison with other days of estrus cycle and anestrus period, it was statistically non-significant. ALT activity was high at the end of estrus cycle in comparison with other days of estrus cycle and anestrus period, these differences were significant (P<0.05).

Key words: Buffaloes, estrus cycle, anestrus, total protein, albumin, globulin, enzymes and cholesterol.

INTRODUCTION

Certain blood serum parameters such as total proteins, albumin. globulin, alkaline phosphatase, transaminase and cholsterol can be measured and used as indication of the reproductive status of buffaloes during the postpartum stage (Badr et al., 2002). Patil and Deshpande (1979) observed that total protein values remained low after parturition in cows which did not show estrus within 90 days but increased significantly in those in estrus. Fahmy et al., (2004) found that the total proteins and its fractions were higher in early conceived than in later conceived cows at 5, 7 and 9 weeks postpartum. Plasma total protein as a constituent of plasma serve as indicator of amino acids pool for protein synthesis in the liver (Harper et al., 1977). Also, Abd-El-Bary (1990) mentioned that plasma total proteins have a particular importance due to the fact that total proteins in plasma generate a colloid osmotic pressure which control the flow of water between blood and tissue fluids. This osmotic effect of plasma proteins depends mainly on the plasma albumin and to a lesser extent on plasma globulin (Harper et at., 1977). Changes in serum transaminase had been reported during different repreoductive stages in cattle (Bugalia et al., 1996). Sharma et al., (1998) and Arosh et al. (1998) mentioned that value of AP was higher in anestrus than in normal cyclic in buffalo heifers and cows respectively. However Kumar and Rattan (1998) observed that AP activity remained stable during estrus cycle in buffalo heifers. Roussel and Staollcup, (1967) observed siginficant differences in transaminase levels between anestrus (lower) and normal cycling cows (higher). The authors found opposite result for alkaline phosphatase value. Parmck and Mehta, (1991) have related the lower serum alkaline phosphatase with the greater ovulation rate in Surti buffaloes. Cholesterol is being the precursor of steroid hormones, its level varied during estrus cycle, where in cattle and buffaloes cholesterol level was higher during estrus compared to anestrus (Sarvaiy and Pathak, 1991, Amanullah *et at.*, 1997 and Singh *et al.*, 1999). Hypercholestraemia was associated with elevated circulatory estrogen profiles during estrus in cycling and nymphomanic (kaushik and Bugalia 1999). This increased level of cholesterol at estrus may be due to withdrawal of stored tissue cholesterol in blood for 17-β estradiol synthesis (Honnappagol and Patil, 1991). This work aimed to study some blood serum constituents which are affected during estrus cycle and anestrus period in Egyptian buffaloes.

MTERIALS and METHODS

Fourteen Egyptian buffaloes from the Experimental farm of the Faculty of Agriculture Al-Azhar University, were used for this study. Seven buffaloes were chosen in estrus and 7 in anestrus period. Animals were fed according to their live body weight and production, on concentrate mixture containing 17% crude protein, 2.5% fat and 15% crude fiber, in addition to rice straw or wheat straw and berssem hay. Egyptian clover, Trifolium alexandrinum, was offered at 20 to 25 kg/animal/day.

Blood samples were collected from the jugular vein of buffaloes with estrus cycle at day of estrus and at two days intervals thereafter for 22 days. Blood samples were collected, only once, from buffaloes in the anestrus period (inactive ovaries for 90 days or more after calving). Buffaloes were detected for estrus signes three times daily, at morning, mid day and afternoon using a separate fertile bull for day of estrus determination, other buffaloes with anestrus were rectally palpated once every week. The serum was separated and stored at- 20°C till determination of the blood serum constituents.

Serum total proteins, albumin, globulin, A/G ratio, alkline phosphatase, aspartate aminotransferase, alanine aminotransferase and cholosterol were estimated. Total protein and albumin were estimated colorimtrically and BCG method in the serum, based on the principle of the Biuret reaction after Weichselbaum (1946) and Gustafsson, (1975)

respectively. Globulin was calculated by subtraction of albumin value from total proteins value. The A/G ratio was calculated. Serum AP was determined colorimetrically by the method of Bessey *et al.* (1946). Serum aspartate aminotransferase and alanine aminotransferase activities were measured as described by king (1965). Serum cholesterol was estimated colorimetrically as described by Frings *et al.* (1972).

Statstical analysis was carried out to find differences of blood constituents amony days of estrus cycle and anestrus period using GLM procedure of SAS (1988).

RESULTS

Total proteins concentration during estrus cycle were significantly (P<0.05) higher at days of estrus, 2, 4 and 22 of estrus cycle than that of anestrus period. No significant differences were found among days 6, 8, 10, 12, 14, 16, and 18 of estrus cycle and anestrus period. Level of total protein was higher at day of estrus, then slightly decreased until day 14 of cycle then slightly increased till the end of the cycle. Level of albumin (Table 1) was high at day of estrus then decreased till day 16 of the cycle then increased at 18, 20 and 22 days of the cycle, these differences were significantly (P<0. 05). The concentration of albumin in anestrus period was lower significantly (P<0. 05) compared with days of estrus cycle. Days of estrus cycle and anestrus period had significant influence (P<0.05) on level of serum globulin (Table 1). The globulin concentration was relatively high at day of estrus and till-10 day from the estrus cycle, reaching the lowest value at 12 and 14 days of estrus cycle, gradually decreased until end of estrus cycle. Globulin values in anestrus were lower significantly (P<0. 05) compared with days of estrus, 2, 4, 6, and 8 from the estrus cycle. The results of the present study showed a significant (P<0.05) variations in the values of A/G ratio during different days of estrus cycle and anestrus period (Table 1). The Value of A/G ratio showed, a slightly decrease from day of estrus until days 10 and 12 of estrus cycle, it had fluctuated increase from day 14 to end of estrus cycle. A/G ratio in anestrus period was similar to days 10 and 12 from estrus cycle.

Changes in serum alkaline phosphatase activity during estrus cycle and anestrus period are shown in Table (2). The level was low at day of estrus then gradually increased till 14 day of estrus cycle. The activity of AP was slightly increased from day 16 of estrus cycle till the end of estrus cycle. Level of AP activity was higher significant (P<0.05)

in anestrus period and at 14 day of estrus cycle compared with the other days of estrus cycle. No Significant differences were found in AST activity throughout the days of estrus cycle and between days of estrus cycle and anestrus period (Table 2). The serum AST activity decreased from day of estrus to 10 day of estrus cycle then increased from day 12 to end of estrus cycle. AST activity in anestrus period was similar to mid of estrus cycle. On the other hand, ALT activity was higher at the end of estrus cycle than at day of estrus. Value of ALT in anestrus period was lower than the end of estrus cycle, these differences among days of estrus cycle and between estrus cycle and anestrus period were significant (P<0.05). Table (2) also showed the changes in the concentration of cholesterol. Level of cholesterol was high at day, of estrus and 2nd of cycle, then steadily declined till day 12 of estrus cycle, then gradually increased till the end of estrus cycle. Concentration of cholesterol in anestrus period was lower significantly (P<0.05) than days of estrus cycle, except day 10 from the cycle. Also, value of cholesterol at 10 days of estrus cycle, was lower significantly (P<0.05) compared with day at estrus and day 2 from estrus cycle.

Table 1: Means ± S.E of serum concentration of total protein albumin, globulin and A/G ratio during estrus cycle and anestrus period in Egyptian buffaloes.

Blood valus Estrus cycle	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
Estrus	8.84± 0.22a	4.32± 0.13a	4.51± 0.09a	0.957± 0.01abc
2	8.70± 0.16a	4.17± 0.09ab	4.52± 0.11a	0.923± 0.02abc
4	8.52± 0.18ab	4.10± 0.07dbc	4.42± 0.12ab	0.928± 0.01abc
6	8.22± 0.14abcd	3.90± 0.04bcd	4.31± 0.11abc	0.906± 0.01bcd
8	7.97± 0.12bcd	3.62± 0.11de	4.30± 0.11abc	0.848± 0.03de
10	7.68± 0.20cd	3.52± 0.10de	4.14± 0.14abcd	0.856± 0.03de
12	7.61± 0.22cd	3.74± 0.12cde	3.87± 0.10d	0.966± 0.007abc
14	7.51± 0.22d	3.68± 0.11de	3.82± 0.11d	0.962± 0.005abc
16	7.64± 0.28cd	3.74± 0.14cde	3.90± 0.13d	0.959± 0.005abc
18	7.92± 0.29bcd	3.91± 0.15bcd	4.01± 0.14cd	0.974± 0.009a
20	8.25± 0.18abcd	3.91± 0.09bcd	4.34± 0.10abc	0.902± 0.01cde
22	8.32± 0.26abc	4.10± 0.13abc	4.22± 0.13abcd	0.969± 0.004ab
Anestrus period	7.52± 0.33d	3.44± 0.17e	4.08± 0.16bcd	0.842± 0.02e

A,b,c,d,e similar superscripts within each column indicate no significant difference between values at P<0.05.

Table 2: Means ± S.E of serum concentration of alkaline phosphatase, asparatate aminotransferase, alanine aminotransferase and cholesterol during estrus cycle and anestrus period in Egyptian buffaloes.

Blood valus	Alkaline phosphatase U/L	AST U/L	ALT U/L	Cholesterol g/dl
Estrus cycle				
Estrus	30.57± 1.13b	35.57± 2.11	21.85± 0.91abc	141.28± 5.41a
2	32.85± 1.76ab	34.28± 2.08	21.42± 0.64abc	140.14± 4.62a
4	32.42±1.39ab	32.85± 2.02	20.85± 0.98bc	137.42± 4.98ab
6	33.14± 1.75ab	31.57± 1.23	20.50± 0.87bc	130.85± 4.69ab
8	34.71± 1.75ab	31.00± 1.75	20.57± 1.13bc	128.42± 3.74ab
10	35.14± 2.16ab	30.00± 1.81	20.28± 1.14c	125.71± 4.57b
12	35.71± 1.96ab	30.85± 1.81	20.71± 1.01bc	127.28± 4.30ab
14	37.42± 2.06a	31.28± 1.92	21.28± 1.34abc	128.57± 3.96ab
16	34.85± 2.07ab	32.42± 2.21	22.00± 0.89abc	129.85± 3.67ab
18	33.28± 1.67ab	31.57± 2.46	22.57± 0.78abc	131.42± 3.56ab
20	32.42± 1.55ab	32.00 ± 2.52	23.71±1.06ab	133.14± 4.31ab
22	32.00± 1.64ab	33.57± 2.43	24.42± 0.99a	136.28± 4.49ab
Anestrus period	36.57± 2.39ab	31.14± 2.09	20.42± 1.13bc	124.57± 2.91b

A,b,c, similar superscripts within each column indicate no significant difference between values at P<0.05. AST: Asparatate aminotransferase, ALT: alanine aminotransferase

DISCUSSION

Changes of total proteins concentration in the present study are in agreement with those found by Patil and Deshpande, (1979) Enkhia et al. (1982), Sarvaiya and Pathak, (1992), Umesh et al. (1995), Burle et al. (1995), Arosh et al. (1998). Singh et al. (1999), Kabir et al. (2001), and Badr et al. (2002) who reported that serum total proteins were higher in normal cycling than in anestrus period or between normal and repeat breeders for cows and buffaloes. Contradictory results were reported by Jo (1981) who found no difference in serum protein concentration among stages of estrus cycle in cows. Also, Shah et al. (2003) showed that the level of total proteins did not differ significantly between fertile and infertile buffaloes at postpartum stages. Cetin et al. (2002) showed that total proteins value were higher in repeat breeder than in fertile cows, this difference was non-significant. This could be due to different animal breeds, health, nutritional and management conditions. Fahmy et al. (2004) mentioned that level of total protein was higher in early

conceived than in later conceived cows, but this difference was not significant. Saleh et al. (1994) reported significant correlation between blood serum composition during estrus and conception which involved serum total protein. Dhaliwal et al. (1990) observed that an optimal protein level was required for estrus expression. Marginally low level of plasma protein was observed in anestrus heifers with inactive and smooth ovaries (Chauhan et al., 1981). Low level of proteins may lead to deficiency of certain amino acids that are required for gonadotrophin synthesis thereby impairing the reproduction (Amanullah et al., 1997).

The results of the present study are in accordance with those observed by Umesh et al. (1995) and Abdul Quayom et al. (1990) who showed that significant difference was observed in the value of total proteins between the day of estrus and anestrus buffaloes. Significant difference was also observed in the level of total serum proteins between the day of estrus and day 10 of the estrus cycle. Also, in buffaloes, Kummar and Rattan, (1998) found that total serum proteins were higher in proesterus and estrus than medestrus. In goat, a sudden drop in total proteins content of Fallopian tube on day 3 of estrus cycle seemed to be due to the effect of lower estradiol levels recorded on the same day (Pathak et al., 1990). Similar findings were recorded during sexual cycles in Fallopian tube of sheep (Rao et al., 1987). The peak levels of total proteins content of Fallopian tube of goat on days 1 and 2 of estrus cycle were referred to peak prolifercative changes occurring under high estradiol levels at estrus (Jaiswial and Mehta, 1991).

The values of albumin obtained in the present work agree with those recorded by Badr et al. (2002) who found that the level of albumin was higher in buffaloes which exhibited estrus than in buffaloes which did not. Fahmy et al. (2004) reported that level of albumin in cow was higher in early conceived cows than in later conceived ones at 9 weeks postpartum. Rowlands et al. (1980) found that cows which were better able to maintain stable albumin concentration were likely to have better fertility. Also, Atallah and Abd-Alla (1998) observed that buffaloes which failed to show cyclic activity till 210 days postpartum had persistently low level of albumin. The authors explained delayed onset of cyclicity after calving to be due to common sequel for inadequate energy balance and/or persistently lower level of albumin around calving. Rowlands et al. (1977) however found in dairy cows no correlation between fertility and albumin concentration at 40 and 100 days postpartum. Also, Saleh et al. (1994) observed nonsignificant difference between mean albumin concentrations during estrus in both

conceived and non conceived lactating buffaloes. On the other hand, Arosh *et al.* (1998) and Cetin *et al.* (2002) reported that albumin level in cows was higher in normal cycling than in anestrus. It is Ibis concluded from this study that there may be consistent relationship between albumin value and exhibit of estrus.

The serum globulin was significantly (P<0.05) lower in anestrus period than in estrus cycle. The results findings are in coincide with the observation of Rowlands et al. (1980) who stated that an increased globulin concentration in the 3 weeks following calving was significantly related to conception rate. Salah et al. (1994) suggested that globulin level was high in buffaloes which conceived in comparison with those failed to conceive. Those buffaloes which became pregnant showed a significant increase in serum globulin level during estrus. Also, they showed that globulin value during estrus was significantly related to conception. Arosh et al. (1998), Badr et al. (2002) and Fahmy et al. (2004) indicated that globulin level was lower in the anestrus than in the normal cyclical and in later conceived than in early conceived buffaloes and cows. Cetin et al. (2002) however found that globulin was lower insignificant in fertile than in repeat breeder cows. The decreased levels of total proteins, albumin and globulin in the present study might have affect the biosynthesis of gondotrophins and gonadal hormones in anestrus buffaloes.

The differences of A/G ratio among days of estrus cycle and anestrus period may be attributed to the differences in albumin and globulin concentrations. Rajora and Pachauri, (1994) showed that A/G ratio was significantly lwo in non-pergnant cows during early lactation than in lactating cows during early mid and late pregnancy. Saleh et al. (1994) observed that mean A/G ratio was higher during estrus in conceived than in not conceived lactating buffaloes. Also, Atallah and Abd-Alla, (1998) stated that buffaloes falied to show cyclic activity till 210 days postpartum persistently lower level of A/G ratio. The authors suggested that delayed onset of cyclicity after calving could be a common sequel for inadequate energy balance and/or persistently lower levels of albumin, globulin and A/G ratio around calving. Fahmy et al. (2004) mentioned that non significant differences were found in A/G ratio between the early and later conceived cows. While, Badr et al. (2002) reported that value of A/G ratio was slightly fluctuated between buffalo which exbitied estrus and did not exhibited estrus during 90 days postpartum.

Alkaline phosphatase activity was high significant in anestrus period and mid of estrus cycle compared to start and end of estrus cycle. AP activity found in the present study are close to with those found by El-Naggar et al. (1993) who reported that AP activity was higher in diesterus than in estrus for both buffalo heifers and native cattle heifers in dry season for later one. The authors mentioned that values obtained for AP in normal cycling animals were significantly higher than those obtained for animals with questionable activity or complete ovarian inactivity. While, Roussel and Stallcup, (1967) reported that serum AP showed no significant change during stages of estrus cycle, values were lower in estrus than in medestrus diestrus. Sharma et al. (1998) mentioned that value of AP was higher in anestrus buffalos heifers than in normal cyclic. Kumar and Rattan, (1998) however observed that AP activity remained stable during estrus cycle in buffalo heifers. Result of the present study are Contrary with that reported by Arosh et al. (1998) who found that AP activity was significantly lower in anestrus than in normal cyclical cows. Also, Badr et al. (2002) stated that AP activity was lower nonsignificant in buffaloes did not exhibit estrus than which exhibit estrus at days 75 and 90 postpartum. Parmar and Mahta, (1991) suggested that the lower serum AP activity was related with the greater ovulatory response in Surti buffaloes. Also, Allcroft and Folley, (1994) found that AP activity was influenced by the change in the levels of estrogen in the blood. In goats, Jaiswal and Mehta, (1991) observed that the AP activity exhibited significant variation between phases of estrus cycle in ipsilateral and contratateral Fallopian tube. The peak of AP activity was recorded on day 3 (medestrus) from the estrus cycle. These findings were close to that obtained in the present study as same trend for AP activity.

Aspartate and alanine aminotransferase obtained in the present study were in accordance with those observed by Roussel and Stallcup, (1967) who found that serum AST and ALT activity reached a peak at the estrus stage which was preceded by a depression at proestrus period, with the lowest level during estrus cycle appearing at diesterus. Also, Badr et al. (2002) indicated that AST and ALT activity were nonsignificant higher in buffaloes which exhibit estrus in comparison with that did not exhibit estrus during 90 days postpartum. Cetin et al. (2002) found that AST and ALT activity were lower insignificant in fertile than in repeat breeder cows. Arosh et al. (1998) however reported that significantly lower activity of AST was observed in anestrus than in normal cyclical cows. The authors mentioned that level of AST is

indicative of level of physiological activity of the tissues. The diminished level of AST might be associated with reduced physiological activity of reproductive organs in anestrus cows. Hormonal imbalance and derange enzymatic action affect the normal reproductive behavior of the animal and cause physiological alteration (Paul et al. 1991). Sharma et al., (1998) mentioned that AST and ALT activities were significantly higher in anestrus buffalo heifers, which confirms the basic principle of increased level of these enzymes during the pathological conditions of the tissue in bovine females (Latif et al., 1993). Also, Gondotra et al. (1993) reported that AST activity was higher in repeat breeder than normal cows and that this condition might indicate the uterine tissue damage in repeat breeder cows. El-Naggar et al. (1983) reported that AST and ALT activities were higher in estrus than diestrus in buffalo cows and in native cattle heifers. The variation in transaminase activities between estrus cycle (high) and anestrus (low) could be attributed to higher level of cortisol as observed during estrus cycle in buffalo heifers by Kumar et al. (1991).

The changes in serum cholesterol concentration with estrus cycle and anestrus period in the present study are conform with those reported by Sarvaiva and Pathak, (1991) Burle et al. (1995), Amanullah et al. (1997), Arosh et al. (1998), Sharma et al. (1998), Kumar an Rattan, (1998), Singh et al. (1999), Kabir et al. (2001) and Badr et al. (2002) who found that cholesterol level was significant and non significant lower in anestrus than in estrus or cycling for both buffaloes and cows. Also, Umesh et al. (1995) observed that significant difference in level of cholesterol between the day of estrus (higher) and day 10 (lower) of the estrus cycle. The results of the present study were not in harmony with that reported by Rowlands et al. (1980) and Saleh et al. (1994) who found that poor fertility was not releated to cholesterol level. Also, Verma et al. (1984) did not observe any significant difference in cholesterol levels of cyclic and acyclic buffaloes. Cetin et al. (2002) however showed that value of cholesterol was higher in repeat breeder than in fertile cows. Pathak et al. (1990) observed that total cholesterol level was positively correlated with blood level of progesterone.

The lower of cholesterol value in anestrus compared with day of estrus may be responsible for the cessation or decrease in gonadal activity affected by the established relationship between the gonadal steroids and blood cholesterol level (Robinson, 1977). Cholesterol is one of the precursors for synthesis of steroid hormones such as androsteindione, progesterone and estrogen (Vohra et al., 1995). The

high level of cholesterol in cyclic animals is indicative of more secretion of steroids during estrus due to increased ovarian activity (Dutta et al., 1989). Sahukar et al. (1985) showed that cholesterol was highest at estrus attributed this to the mechanism by which estrogens affected the complex inter-relationship of pituitary-thyroid-adrenal functions. Elevation of cholesterol at estrus was associated with increased endogenous biosynthesis of cholesterol rather than to a difference in the rate of catabolism. Also, Honnappogol and Patil, (1991) reported that increased level of cholesterol at estrus may be due withdrawal of stored tissue cholesterol in the blood for $17-\beta$ estradiol synthesis.

It is concluded from this study that changed concentration of certain blood profiles might be responsible for the production of acyclicity in postpartum buffaloes.

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