PLASMA CHEMICAL COMPOSITION AND PROGESTERONE HORMONE ON DAY OF ESTRUS IN EGYPTIAN BUFFALO COWS

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ABSTRACT

The aim of this study was investigate concentrations of blood plasma biochemical composition, minerals and progesterone on the day of estrus for buffalo cows in Minoufiya province in Egypt.

The current study was carried out in the veterinary units on 33 Egyptian buffalo cows that were chosen randomly on day of estrus and from 13 normal postpartum dairy buffalo cows (as a control group) that were chosen after about 6 to 12 h from parturition. In our study, initial blood samples were taken at the time of AI which was within the first 12 h between estrus and ovulation and hence all animals were at the same stage of the estrous cycle.

Concentrations of P4, CH and AL/GL ratio significantly ($P<0.05$) increased in normal cows than those in estrus but only G, TP, AL, and GL concentrations were significantly ($P<0.05$) higher in case of estrus than in normal. The normal plasma concentrations of Mg, Mn, Se, Fe, Mo, and Cd in buffalo cows were higher than in estrus day. On the other hand, the blood plasma concentrations of Na, K, Ca, P, Ca/P ratio, Co, Cu, and Zn were higher in estrus than normal.

Progesterone concentrations were higher on day of estrus in winter than in summer. All plasma macro elements were higher on day of estrus in winter than in summer except Ca, P and Ca/P ratio. On the other hand, all plasma macro elements were higher on day of estrus in winter than in summer except Zn, Mn, Se, and Fe.

Keywords: buffalo, progesterone, glucose, cholesterol, macro-minerals, micro-minerals, estrus, plasma

INTRODUCTION

Buffalo, the main dairy animal in Egypt, was known as a late maturing. Most literatures indicated that average age at first calving was about 40 months. This is influenced mainly by the interval from calving to subsequent conception and is turn influenced by the time taken after calving for involution of the uterus, reestablishment of cyclic ovarian activity and return to normal fertility. The delay of first postpartum ovulation and estrus is considered the major factor lead in to a long calving to conception period in Egyptian buffaloes (El-Fouly, 1983). In general, buffaloes are characterized by poor reproductive performance as compared to cattle (Gordon, 1996).

Reproduction is one of the key pillars of dairy production. Many dairy herds do not

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achieve their targets for reproductive performance and incur substantial economic opportunity cost. Despite the fact that postpartum uterine disease is only one component of reproductive performance, and that it generally has secondary importance to insemination efficiency (LeBlanc, 2005), it has traditionally occupied a substantial amount of veterinarians' attention.

Minerals are the essential nutrients bearing a significant role in the animal reproduction, because their excess or deficiency produces detrimental effect on the performance of livestock. Trace elements including Cu, Co, Zn, Fe, Se, I, Mo, Mn and certain macro-elements like K, Ca, Na, Cl, P have been found to be very essential for normal livestock growth (Underwood, 1981). Trace elements may function as cofactors, as activators of enzymes, or as stabilizers of secondary molecular structure (Valee and Wacker, 1976). Deficiency or excess of minerals like P, Cu and Zn have been associated with subnormal fertility and anoestrus conditions (Moddie, 1965). It was hypothesized that the deficiency or excess of some micro minerals in the blood may cause anoestrus in buffaloes. The present study was, therefore, designed to determine levels of micro minerals (Cu, Fe, Zn, Se) in the serum of cyclic and anoestrus Nili-Ravi buffaloes.

MATERIALS AND METHODS

The present study was carried out in veterinary units in Menufiya, Egypt during one year from May 2012 to April 2013.

Animals

Thirty three postpartum Egyptian buffalo cows having 4 to 9 years, 345 to 620 LBW and within the 2 to 6 parity were subjected in this study. The experimental cows included 13 normal buffaloes.

Feeding and housing system

Some subjected buffaloes were fed rations consisted of a commercial concentrate feed mixture (60% yellow corn, 15% wheat bran, 23% soybean meal, 1% Na CL and 1% mineral mixtures), green clover (*Trifolium alexandrinum*) and rice straw, while others were fed bran and green clover and rice straw. Animals were milked twice daily morning and evening milking.

Blood samples and chemical analysis

Blood biochemical and minerals assay

Blood samples were collected from the jugular vein of all buffaloes. Blood samples were collected into clean heparinised test tubes and transported to the laboratory, centrifuged at 3000 rpm for 20 minutes and blood plasma was carefully separated and stored frozen at -20°C until time of chemical analyses.

Blood plasma was carefully digested by adding 10 ml concentrate H_2SO_4 and two drops of H_2O_2 to 1 ml of blood plasma and heated. The digested sample was diluted with distillated water at a ratio of 1:50. Concentrations of macro- (Na, K, Mg, and Ca) and micro- (Mn, Cd, Se, Co, Cu, Mo, Fe, and Zn) elements were determined in blood plasma using an atomic absorption spectrophotometer (Unicam 929 AA). A standard ICP-OES (Perkin-Elmer, Optima 2000 DV) analyzer system was used to the determination according to (Oser, 1965). However, concentration of cholesterol, glucose, total protein, albumin, and inorganic phosphorus (Dryer, 1957) was determined calorimetrically. However, concentration of globulin was calculated by subtracting albumin from total protein concentration.

Progesterone hormone assay

Progesterone was measured in plasma by the radioimmunoassay (RIA) technique using the coated tube kits according to Haynes *et al*. (1980); Blight and White (1983). The kit was purchased from Institute of Isotopes Co. Budapest and was labeled with I^{125}. The tubes were counted in the laboratory of radioactive using computerized Gamma Counter (Packard Instrument Company).

The standard curve of P4 ranged between 0.0 and 37.7 ng/ml. All reagents were equilibrated to room temperature. Then duplicate tubes were labeled for each of total counts (T), non-specific binding (NSB) zero standard (standard 1=B0), standards (S2-6), control (C) and either milk or plasma samples (S). The reagents and samples were mixed thoroughly before used, then 50 µl from each of standards, control and samples were piptted into the properly labeled tubes. Thereafter 100 µl of tracer solution and 100 µl of antiserum were piptted into all tubes except T and NSB, which were antiserum free. All tubes were vortex mixed thoroughly for 2 to 5 seconds except T. The tubes were incubated for 2 h. at room temperature (20 to 28˚C), and then they were placed on a separate tube rack. A bottle containing magnetic immunosorbant (MIS) was shacked and swirled gently until homogeneity then 500 µl was added to each tube except T. All tubes were vortex mixed thoroughly and incubated for 15 minutes at room temperature. The bound fraction was magnetically separated. The rack was attached onto the magnetic separator base and the MIS particles were settled for 5 minutes. The supernatant were poured off and discarded. The tubes were placed on a pad of absorbent tissue and allowed to draining for 2 minutes. The bound fraction was also separated by centrifugation for 15 minutes at 1,500 xg. The radioactivity of all tubes was counted preferably not less than 60 seconds. The assay protocol is shown in Table A.

Statistical analysis

Data obtained were statistically analyzed

Table A. The assay protocol of progesterone.

Tubes reagents	Total count (T)	Non-specific binding (NSB)	Standard (S1-6)	Sample (Sx)	Control (C)
Standard solution 1-6, µl	-	-	50	-	-
Sample, µl	-	-	-	50	
Control, µl	-	-	-	-	50
Tracer, µl	100	100	100	100	100
Antiserum, µl	-	-	100	100	100
Vortex mix. Incubate for 2 h at room temperature, 20 to 28˚C,					
Magnetic immuno-sorbant, µl	-	500	500	500	500
Vortex mix. Incubate for 15 minutes at room temperature, 20 to 28˚C,					
Magnetic separation for 5 minutes or centrifuge for 15 minutes at 1500 xg,					
Removing the supernatant and blot the tubes,					
Count all tubes.					

The standard curve of progesterone ranged between 0.0 and 37.7 ng/ml.

using computer program of SAS (SAS, 2004) (Descriptive statistics, ANOVA and Duncan test was used to determined the significant differences among means at α=0.05).

RESULTS AND DISCUSSION

I: Blood Biochemistry and Progesterone concentration

A: Progesterone and Biochemical components

Concentration of some blood biochemical components including cholesterol (CH), glucose (G), total protein (TP), albumin (AL), globulin (GL), AL/GL ratio and Progesterone (P4) concentration in plasma of normal cows and those at time of estrus are depicted in Table 1. Concentration of P4, CH and AL/GL ratio significantly (P<0.05) increased in normal cows than those in estrus but only G, TP, AL, and GL concentrations were significantly (P<0.05) higher in case of estrus than in normal.

In contrast, dairy animals with higher plasma cholesterol are more likely to express estrus as lipids are the precursors of gonadal steroid hormones (Jorritsma *et al*. (2003). *Zaman et al*. (1985) reported a non significant difference in levels of plasma cholesterol of cyclic and non-cyclic buffaloes. Majeed *et al*. (1990) found a non significant difference in serum cholesterol level between endometritic and healthy buffaloes

Takkar *et al*. (1983) reported that progesterone levels were 0.360±0.062 and 0.334±0.066 ng/ml on the day of estrus in buffalo-heifers and buffalo cows, respectively. The values were around 1 ng/ml till day 6, followed by a gradual increase to a peak average value of

Table 1. Concentrations of P4 and blood biochemical components in normal and estrus buffalo cows.

Items	Case	Mean ±SE		Min	Max
Progesterone concentration:					
P4 conc.(ng/ml)	Normal	0.36	±0.001	0.35	0.38
	Estrus	0.33	±0.002	0.32	0.34
Blood biochemical components:					
Cholesterol (mg/dl)	Normal	198.82	±1.37	189.30	204.50
	Estrus	216.90	±6.93	131.71	259.37
Glucose (mg/dl)	Normal	73.76	±0.86	70.35	79.78
	Estrus	66.75	±2.29	44.64	89.29
Total protein (mg/dl)	Normal	6.83	±0.15	6.21	7.94
	Estrus	6.73	±0.12	5.26	7.59
Albumin (mg/dl)	Normal	5.78	±0.12	5.38	6.50
	Estrus	5.34	±0.16	3.68	7.59
Globulin (mg/dl)	Normal	1.05	±0.09	0.70	1.64
	Estrus	1.39	±0.12	0.02	2.47
A/G Ratio	Normal	5.97[a]	±0.47	3.72	8.29
	Estrus	1.28[b]	±0.03	1.00	1.67

Normal (N=13), Estrus (N=33)

4.888±0.399 and 5.119±0.415 ng/ml on day 15 of the cycle in heifers and cows, respectively. Thereafter, the progesterone concentration fell abruptly to a level similar to that at estrus. The mean progesterone value a day before estrus was 0.488±0.067 and 0.577±0.053 ng/ml in buffalo-heifers and buffalo-cows, respectively. The mean progesterone concentration of different days of the cycle (except day 16) did not differ significantly (P<0.01) between heifers and cows.

On the other hand, Kanai *et al*. (1984) conducted that Progesterone levels began to increase after Day 5 (Day 0 = the day of estrus) and reached a plateau after Day 10. A rapid decrease in progesterone levels occurred during the 5 days before estrus, followed by a sustained increase in estradiol concentrations. Basal LH levels decreased towards the mid-luteal phase and then progressively increased during the follicular phase.

The concentration of progesterone was lowest on the day of insemination, and increased significantly to a peak level of 4.00±0.60 ng/ml by day 13 post insemination. After day 17, it declined significantly (P<0.01) to reach low levels by day 21 (Arora *et al*., 1982).

B: Macro-elements and micro-elements

Concentration of some blood macro-elements and micro-elements in plasma of normal cows and those with reproductive disorders are shown in Table 2a and 2b.

The normal plasma concentrations of Mg, Mn, Se, Fe, Mo, and Cd in buffalo cows were higher than in estrus day. On the other hand, the blood plasma concentrations of Na, K, Ca, P, Ca/P ratio, Co, Cu, and Zn were higher in estrus than normal.

The mean serum copper level in cyclic buffaloes was 70.59±2.59 µg/dl vs 62.23±2.20 µg/dl in anoestrus buffaloes, the difference being significant (P<0.05). Lower copper concentration in anoestrus cattle has been reported in various studies (Deshpande *et al*., 1981; Dabas *et al*., 1987; Dutta *et al*., 2001).

Estrogen hormone has been reported to increase copper level (Sato and Henkin, 1973) and the lower level of copper in anoestrus buffaloes in the present study may be due to lower estrogen level in anoestrus animals (Rajkumar *et al*., 2006). Mean serum iron contents in cyclic and anoestrus buffaloes were 370±2.88 and 358.13±3.46 µg/

Table 2a. Blood plasma macro elements in normal and estrus buffalo cows.

Case	Macro	Mean±SE (mg/dl)		Min	Max	Macro	Mean±SE (mg/dl)		Min	Max
Normal	Na	146.26[b]	±0.67	143.0	150.0	Ca	10.39[a]	±0.29	9.16	12.26
Estrus		173.58[a]	±6.92	102.9	214.1		11.33[a]	±0.32	8.50	15.16
Normal	K	6.13[b]	±0.31	4.90	7.80	P	5.22[a]	±0.17	4.45	6.20
Estrus		24.[36a]	±2.09	4.96	38.62		5.69[a]	±0.17	4.09	7.39
Normal	Mg	5.22[a]	±0.08	1.41	6.53	Ca/P Ratio	1.99[a]	±0.02	1.91	2.08
Estrus		4.55[a]	±0.31	4.93	5.73		2.08[a]	±0.11	1.15	3.43

Normal (N=13), Estrus (N=33)

Table 2b. Blood plasma micro elements in normal and estrus buffalo cows.

Case	Micro	Mean±SE (µg /dl)		Min	Max	Micro	Mean±SE (µg /dl)		Min	Max
Normal	CO	3.08[a]	±0.21	2.14	4.78	Se	0.69[a]	±0.02	0.66	0.84
Estrus		3.14[a]	±0.20	0.69	5.14		0.34[b]	±0.03	0.03	0.74
Normal	CU	0.92[a]	±0.05	0.65	1.15	Fe	4.10[a]	±0.16	3.36	4.87
Estrus		0.99[a]	±0.09	0.05	1.90		2.37[b]	±0.38	0.32	9.00
Normal	ZN	0.85[a]	±0.09	0.44	1.50	Mo	0.13[a]	±0.002	0.12	0.15
Estrus		1.14[a]	±0.41	0.20	9.99		0.04[b]	±0.002	0.02	0.08
Normal	MN	0.29[a]	±0.03	0.16	0.47	Cd	1.13[a]	±0.04	1.08	1.60
Estrus		0.21[a]	±0.03	0.05	0.85		0.89[a]	±0.07	0.19	1.60

Normal (N=13), Estrus (N=33)

dl, respectively, the difference was significant (P<0.05), Dutta *et al*. (2001). Lower level of iron causes normochromic anemia in animals, which in turn affects the response of ovarian receptors to estrogen, resulting in anoestrus condition in animals (Kumar and Sharma, 1993). Mean serum zinc level in cyclic buffaloes was significantly higher (181.4±2.35 µg/dl) than in anoestrus buffaloes (164.07±2.01 µg/dl). Dutta *et al*. (2001) also reported low zinc level in anoestrus heifers.

Fall in zinc level was associated with fall in steroid hormone concentrations which indicated that there was some co-relations between plasma zinc levels and progesterone-estrogen levels for proper reproductive processes. Selenium level in the cyclic buffaloes was 0.10±0.008 µg/ml and in anoestrus buffaloes it dropped significantly to 0.07±0.010 µg/ml. In cattle and sheep, selenium deficiency is associated with reduced fertility (Hidiroglou, 1979) and high selenium concentration educes the incidence of anoestrus (Harrison *et al*., 1984). It was concluded that the deficiencies of copper, iron, zinc and selenium either singly or in combination could be responsible for anoestrus condition in Nili- Ravi buffaloes and by improving the nutritional status the fertility can be improved in females of this species.

The mean serum copper level in cyclic buffaloes as 70.59±2.59 µg/dl vs 62.23±2.20 µg/dl in anoestrus buffaloes, the difference being significant (P<0.05). Lower copper concentration in anoestrus cattle has been reported in various studies (Deshpande *et al*., 1981; Dabas *et al*., 1987 and Dutta *et al*., 2001).

Mean serum iron contents in cyclic and anoestrus buffaloes were 370±2.88 and 358.13±3.46 µg/dl, respectively, the difference was significant (P<0.05), Dutta *et al*. (2001). Mean serum zinc level in cyclic buffaloes was significantly higher (181.4±2.35 µg/dl) than in anoestrus buffaloes (164.07±2.01 µg/dl), Dutta *et al*. (2001). Selenium level in the cyclic buffaloes was 0.10±0.008 µg/ml and in anoestrus buffaloes it dropped significantly to 0.07±0.010 µg/ml (Hidiroglou, 1979) and high selenium concentration reduces the incidence of anoestrus (Harrison *et al*., 1984).

II: Effect of season

Blood biochemistry

Season changes of P4 concentrations and some plasma biochemical components at estrus time are depicted in Table 3.

This study represents that Progesterone concentrations were higher on day of estrus in winter than in summer and these results is agree with Rao *et al*. (1982) who reported that Plasma progesterone concentrations on the day of estrus and also in the luteal phase of the cycle were significantly higher ($P<0.01$) in the cooler (warm and cold) seasons than in the hot and dry and hot and humid seasons.

Also, all blood biochemical components were higher on day of estrus in winter than in summer except globulin (GL) and AL/GL ratio.

Macro-elements and micro-elements

The seasonal variations of some plasma macro and micro elements concentration at estrus time are depicted in Table 4a and 4b.

In this respect, all plasma macro elements were higher on day of estrus in winter than in summer except Ca, P and Ca/P ratio. On the other hand, all plasma macro elements were higher on day of estrus in winter than in summer except Zn, Mn, Se, and Fe.

Table 3. Seasonal variations of blood biochemical components in buffalo cows.

Items	Normal		Estrus	
	Summer	Winter	Summer	Winter
P4 conc.$_{(ng/ml)}$	0.33a±0.003	0.33a±0.002	0.35a±0.002	0.36a±0.002
Cholesterol$_{(mg/dl)}$	200.20a±0.46	198.20a±1.97	184.42b±11.26	240.83a±2.45
Glucose$_{(mg/dl)}$	72.14a±0.51	74.47a±1.16	60.85b±4.34	71.09a±1.93
Total protein$_{(g/dl)}$	6.69a±0.09	6.89a±0.21	6.47b±0.21	6.92a±0.11
Albumin$_{(g/dl)}$	5.49b±0.10	5.91a±0.15	5.01b±0.33	5.59a±0.14
Globulin$_{(g/dl)}$	1.19a±0.18	0.98a±0.09	1.46a±0.19	1.33a±0.15
Alb/Glob Ratio	5.08b±1.09	6.36a±0.47	1.33a±0.06	1.25a±0.03

Table 4a. Seasonal variations of plasma macro elements in buffalo cows.

Items (mg/dl)	Normal		Estrus	
	Summer	Winter	Summer	Winter
Na	146.14a±1.26	146.31a±0.84	144.45b±9.05	195.05a±6.62
K	6.00a±0.61	6.19a±0.39	12.89b±2.49	32.82a±0.89
Mg	5.07a±0.11	5.29a±0.10	2.88b±0.21	5.78a±0.27
Ca	10.08a±0.32	10.53a±0.40	12.11a±0.59	10.75b±0.29
P	5.14a±0.18	5.25a±0.24	5.81a±0.33	5.59b±0.18
Ca/P Ratio	1.96a±0.02	2.01a±0.02	2.22a±0.19	1.98a±0.11

Table 4b. Seasonal variations of plasma micro elements on the day of estrus in buffalo cows.

Items (µg /dl)	Normal		Estrus	
	Summer	Winter	Summer	Winter
Co	3.09a±0.31	3.07a±0.28	2.76±0.41	3.41±0.17
Cu	1.003a±0.08	0.88a±0.07	0.71b±0.19	1.19a±0.07
Zn	0.77a±0.04	0.79a±0.12	2.11a±0.91	0.42b±0.05
Mn	0.36a±0.05	0.27b±0.04	0.29a±0.06	0.15b±0.03
Se	0.68a±0.005	0.71a±0.03	0.41a±0.04	0.29b±0.04
Fe	3.98a±0.29	4.16a±0.19	3.86a±0.69	1.27b±0.21
Mo	0.14a±0.0002	0.14a±0.003	0.03b±0.004	0.05a±0.002
Cd	1.12a±0.02	1.14a±0.06	0.52b±0.09	1.16a±0.06

CONCLUSIONS

Blood samples were taken from 33 buffalo cows that was chosen randomly on day of estrus and from 13 normal postpartum dairy buffalo cows (as a control group) that were chosen after about 6 to 12 h from parturition. This permitted us to measure serum mineral concentrations directly related to estrus and normal. Results show that concentration of CH and AL/GL ratio significantly ($P<0.05$) increased in normal cows than those in estrus but only G, TP, AL, and GL concentrations were significantly ($P<0.05$) higher in case of estrus than in normal.

The normal plasma concentrations of Mg, Mn, Se, Fe, Mo, and Cd in buffalo cows were higher in normal than in estrus group. On the other hand, the blood plasma concentrations of Na, K, Ca, P, Ca/P ratio, Co, Cu, and Zn were higher in estrus than normal.

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