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RELATIONSHIPS BETWEEN SERUM PROTEINS AND HORMONAL PROFILE IN BUFFALOES WITH NORMAL AND DISTURBED OVARIAN ACTIVITY

MONA M. ALAA EL-DIN; M. S. HASSAN**; SAMIRA A. EMARA*
and KARIMAN D. HASSANIN

Physiology Unit, Biology Department - ARRI
*Biochemistry Unit, Biology Department - ARRI
**Theriogenology Department, Faculty of Vet. Med. - Cairo University

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SUMMARY

The aim of this study was to investigate the correlation between serum protein and its electrophoretic pattern (albumin and globulin fractions) as well as the hormonal profile (estradiol 17B and progesterone) in normal cycling buffaloes during the follicular and luteal phases of oestrous cycle and in those affected with ovarian inactivity. The study was carried out on 12 apparently normal buffaloes (5 cycling and 7 suffering from ovarian inactivity). In the cycling animals, blood samples (10 ml) were collected daily starting from day 0 of the cycle till day 6 then every other day till day 22, while in the animals suffering from inactive ovary, 5 samples were collected with 3 day-intervals. The serum was separated and stored at - 20°C until assayed for total protein , electrophoretic pattern as well as for estradiol 17B and progesterone.

A significant decrease of α globulin and A/G ratio were observed in animals suffering from ovarian inactivity. Meanwhile, α globulin increased significantly in these animals in comparison with the cycling ones. On the other hand, total protein, β globulin and albumin were not significantly changed. However, in the cycling animals, total protein and albumin significantly increased during the follicular phase of the oestrous cycle.

In conclusion, determination of some serum biochemical parameters such as serum proteins could be of value in prediction of some infertility problems in the buffalo.

INTRODUCTION

The ability of buffalo to thrive under tropical conditions, to utilize coarse roughage, to provide

a good yield of milk with high fat content and to be a popular with the farmers are the best incentives for buffalo breeding. Ovarian inactivity is a major cause of reproductive disturbances in buffaloes. Its incidence in abattoir surveys ranges from 3 to 39% (Dwivedi and Singh,1971 and Aggag,1986). According to Rao and Sreemanrayana (1982), the inherently suboptimal functioning of hypothalamo-hypophysial-gonadal axis and consequent low circulating hormones might be responsible for higher incidence of inactive ovaries and true anoestrus in buffaloes. Clinical surveys data revealed higher incidence of anoestrus and inactive ovaries in buffaloes (55.5% and 19.4% respectively) (El-Naggar and El-Sherry, 1974).

Protein is the most fundamental component of animal tissues and a continuos supply of it, is needed throughout life. It is essential for tissue repair, growth and formation of animal products. Moreover, globulins, particularly Igs are known to play an important role in host protection against invading pathogens through initiation /amplification of defense mechanism. A shortage of protein was claimed to be one of the major causes of infertility in the female (Bondi,1987). Moreover, all enzymes and certain hormones are proteinous in nature (Muthe,1981 and Stryer, 1981).

As regard to the relationship between total serum protein, albumin, globulins and fertility, Rowlands et al.(1977) observed that serum albumin was higher in cattle that conceived after 4 or more services, while serum globulins were higher in cattle that conceived after more than one service compared to of normal cattle. Rowlands and Pocock (1976) mentioned that the fertility of cows tend to be reduced if albumin concentration fell below 3.2 gm%. Significant decrease in serum protein was recorded in cattle and buffaloes suffering from inactive ovaries. These changes were attributed to insufficient diet or poor absorption of diatary constituents from intestinal tract (El-Baghdady, 1979; Abdel-Rehim,1982; Farrag and Hassan, 1982) or hormonal imbalance, particularly, gonadotrophins (El-Azab et al., 1988). On the contrary, some researchers reported no significant differences between the values of serum protein, albumin and globulins in normal cyclic buffaloes and those affected with inactive ovaries (Mikhail, 1979; Samad et al., 1980; Moustafa et al., 1988).

Thus, the aim of this study was to investigate the correlation between serum protein and its electrophoretic pattern (albumin and globulin fractions) as well as the hormonal profile (estradiol 17B and progesterone) in normal cycling buffaloes during the follicular and luteal phases of oestrous cycle and in those affected with ovarian inactivity.

MATERIAL AND METHODS

This study was carried out on 12 buffaloes (5 cycling and 7 suffering from ovarian inactivity). The animals aged 4 - 8 years and were kept at Animal Reproduction Research Institute during the Spring,1998.The animals were apparently healthy, free from parasitic and infectious diseases and fed on standard balanced ration.

Behavioral estrus was checked daily with the help of a buffalo-bull and depending on the estrus signs observed. Rectal examination was used to ensure estrus and to diagnose the cases of ovarian inactivity. In the cycling animals, the day of estrus was considered as day zero.

In the cycling animals, blood samples (10 ml) were collected daily starting from day 0 of the cycle till day 6 then every other day till day 22, while in the animals suffering from inactive ovaries, 5 samples were collected with 3 day-intervals. Blood samples were immediately centrifuged at 3000 r.p.m. for 10 minutes at 4°C. The serum was separated and stored at - 20°C until assay.

Hormonal assay:

Progesterone values were determined using RIA. Direct progesterone coated tubes were used in the assay (Diagnostic products corporation ,USA).The sensitivity of the assay was 0.05 ng/ml. Estradiol 17B levels were determined using double antibody RIA kits (Diagnostic systems laboratories, USA). The sensitivity of the assay was 0.5 pg/ml.

Serum proteins

a) Total protein:

Serum total protein was determined by the Biuret reaction according to Weichsallaum (1946) where alkaline copper reacts with peptide bond in protein forming a violet color.

b)Electrophoretic pattern of serum protein:

The method used for electrophoretic separation of serum proteins was based on the separation of charged molecules according to their movement under the influence of an applied electric field (Melvin, 1987). Serum samples were applied to the buffered cellulose acetate strips, after electrophoresis. The strips were removed carefully, immersed in fixative dye solution. Destaining was applied to remove excess of stain, then strips were fixed on a special glass plate and immersed on clearing solution, dried and quantitated by scanning (Melvin,1987 and Kaneko, 1989).

c) Albumin/globulin (A/G) ratio:

Albumin / Globulin ratio was calculated according to the results obtained from the electrophoretic separation of serum protein.

Statistical analysis:

Statistical analysis of data (t-test and F-test) were done using Microsoft Excel 97, version 6.0.

RESULTS

The results of this study were given in tables 1,2 and summarized in table 3. It is clear that total protein values during the follicular phase (8.02±0.67 gm) were significantly higher ($P< 0.05$) than those observed during luteal phase (6.6±0.25 gm) while there was no significant difference in total protein values in cycling animals compared to those suffering from inactive ovaries, although the values tended to be lower in

Table 1: Values (Means ± SE) of serum protein and its electrophoretic pattern, Estradiol 17-ь and Progesterone in the cycling buffaloes (n=5 animals).

Days of the estrous cycle	Total protein (gm)/100ml	Albumin		A/G ratio	globulin		globulin		globulin		Estradiol - 17- pg/ml)	Progesterone ng/ml
		gm	%		gm	%	gm	%	gm	%		
Day 0 (estrus)	10.4+1.3	4.3+0.8	51.1+3.2	0.70	1.7+0.8	16.1+2.3	2.2+1.2	20.9+2.3	3.39+1.2	38.6+3.5	22.4+3.4	0.2+0.2
1	7.26±1.2	2.99±1.2	32.3±4.6	0.71	1.5±1.0	17±2.6	1.12±0.8	12.1±2.1	0.57±0.2	6.1±4.2	10.3±4.2	0.5±0.3
2	7.02±1.8	2.84±2.1	40.4±4.2	0.66	1.71±0.8	24.3±3.4	0.77±0.2	11±2.5	1.69±0.8	23.1±2.8	4.18±2.6	0.6±0.2
3	7.78±1.1	2.75±1.8	35.4±2.2	0.55	1.6±1.2	20.6±3.2	1.35±0.8	17.2±2.3	2.08±1.2	26.7±3.4	3.85±1.2	0.9±0.2
4	7.54±1.3	1.96±1.2	22.3±2.3	0.33	2.44±1.4	32.3±2.5	0.65±0.2	8.6±2.1	3.05±1.4	36.8±3.6	3.6±1.8	1.1±0.4
5	5.96±2.1	1.49±1.1	24.1±3.6	0.33	2.16±1.6	36.2±2.2	1.28±0.8	9.37±2.2	1.02±0.4	17.1±4.1	3.8±1.9	1.3±0.3
6	6.6±1.7	1.9±0.8	28.6±3.8	0.42	0.9±1.5	13±4.3	1.7±0.6	26.2±2.5	2.1±1.2	32.2±3.5	3.6±2.3	1.6±0.3
8	5.86±1.6	1.7±1.2	29.22±2.2	0.41	1.4±0.6	24±2.5	0.69±0.5	11.8±3.4	2.06±1.4	35.3±2.8	2.9±1.2	1.9±0.8
10	5.51±1.6	1.43±0.9	25.9±2.1	0.34	1.19±0.8	21.6±5.1	0.95±0.4	17.2±2.1	1.94±1.3	35.2±2.3	3.3±2.1	2.1±0.4
12	7.08±1.3	2.48±1.8	35.1±3.2	0.53	1.19±1.1	16.8±3.2	1.84±0.8	25.9±2.5	1.57±0.8	22.2±3.4	3.8±1.8	2.4±0.8
14	6.38±0.9	1.76±1.6	27.5±2.1	0.38	1.28±1.2	20±4.6	1.01±0.6	15.8±2.1	2.35±1.4		3.13±15	2.8±0.6
16	5.84±1.5	1.41±2.1	24.1±3.8	0.32	1.9±0.8	23.5±4.1	1.3±0.8	22.4±2.4	1.14±0.6	9.6±6.2	6.16±3.2	1.8±0.6
18	8.06±1.4	2.11±1.4	26.2±3.2	0.35	2.51±1.3	43±2.8	1.54±0.8	19.1±3.5	1.43±0.7	31.3±4.2	12.3±4.5	0.9±0.4
20	7.25±2.1	2.22±1.6	30.7±2.8	0.44	1.4±1.4	16.6±5.1	1.94±0.6	31.2±2.1	1.07±0.6	26.1±4.0	16.7±6.2	0.6±0.3
22	9.32±2.4	4.53±1.8	61.9±5.2	0.94	1.58±1.2	20.5±4.2	0.84±0.4	11.4±3.4	2.3±1.2	21.9±3.8	18.5±5.6	0.5±0.2

Table 2: Values (Means ± SE) of serum protein and its electrophoretic pattern, Estradiol 17-ь and Progesterone in the buffaloes suffering from ovarian inactivity (n = 7 animals).

	Total protein (gm)/ 100ml	Albumin		A/G ratio	globulin		globulin		globulin		Estradiol - 17- pg/ml)	Progesterone ng/ml
		gm	%		gm	%	gm	%	gm	%		
1st sample	4.72±2.3	1.7±2.1	48.2±5.1	0.56	2.11±1.4	15.9±3.6	0.75±0.4	14.5±2.5	1.7±0.6	21.3±3.5	1.5±0.06	0.0±0.0
2nd sample	5.7±2.1	1.28±1.4	22.4±6.2	0.28	1.12±1.6	19.7±4.2	1.12±0.8	19.6±2.4	2.18±1.1	38.3±4.1	1.2±0.04	0.1±0.02
3rd sample	6.63±1.8	2.29±1.8	34.5±4.3	0.52	1.36±1.4	20.5±4.6	0.64±0.2	9.7±3.0	2.35±1.2	35.2±3.8	1.3±0.04	0.15±0.03
4th sample	6.8±2.6	1.6±1.3	22.9±4.4	0.30	0.6±1.3	9.2±8.2	1.2±0.5	17±2.8	3.4±1.2	50.9±4.6	1.2±0.05	0.11±0.02
5th sample	7.2±1.9	1.3±1.4	17.5±5.2	0.22	1.6±1.2	22.5±4.8	0.2±0.6	3.3±2.1	4.1±1.4	56.7±4.2	1.1±0.06	0.0±0.0

case of animals suffering from inactive ovary.

Concerning albumin, a significant decrease (P< 0.05) was recorded during the luteal phase versus follicular phase (29.8±2.11% vs 37.7±2.51%). Meanwhile. Non significant decrease was observed in animals suffering from inactive ovary versus cycling animals (29.1±5.53% vs 32.9+2.81%).

Alpha, beta and gamma globulins showed non significant differences during the follicular and luteal phases of oestrous cycle. Meanwhile, α and β globulins were significantly decreased (P<0.05) in animals suffering from inactive ovaries versus cycling animals (17.56±2.34% vs 23.03±2.12% and 12.82±2.88% vs 17.34±1.77% respectively). On the contrary, α globulin fraction was significantly increased (P< 0.05) in animals suffering from inactive ovaries versus cycling animals (40.48±6.21% vs 25.87±2.66%).

Estradiol17B and progesterone were significantly lower (P< 0.002 and P< 0.02 respectively) in animals suffering from inactive ovary versus cycling animals (1.26±0.06 pg/ml vs 7.91±1.68 pg/ml and 0.07±0.03 ng/ml vs 1.28±0.2 ng/ml respectively).

The observed changes in the fractions of serum proteins had resulted in a significant decrease in the values of A/G ratio (P<0.05) in animals suffering from ovarian inactivity in comparison with that reported in the cycling animals (0.37±0.06 vs 0.52±0.04).

Table 3: Summary of the results (Means ± SE) and statistical significance in cycling animals and those affected with ovarian inactivity.

Total protein (gm)/ 100 ml	Cycling animals	Inactive ovaries	Significant difference	Estrous cycle		
				Follicular phase	Luteal phase	Significant difference
	7.19±0.3	6.2±0.4	NS	8.02±0.6	6.6±0.2	NS
Albumin %	32.9±2.8	29.1±5.5	NS	37.7±2.5	29.8±2.1	(P<0.05)
A/G ratio	0.52±0.04	0.37±0.06	(P<0.05)	0.58±0.1	0.44±0.03	NS
α globulin %	23.03±2.1	17.56±2.3	(P<0.05)	22.7±2.3	23.2±2.5	NS
β globulin%	17.34±1.7	21.82±2.8	NS	19.5±1.82	15.8±2.2	NS
α globulin %	25.87±2.6	40.4±6.2	(P<0.05)	22.2±3.6	28.5±3.1	NS
Estradiol 17-β (pp/ml)	7.9±1.6	1.2±0.06	(P<0.002)	22.2±3.6	3.57±1.6	(P<0.003)
Progesterone ng/ml	1.28±0.2	0.07±0.03	(P<0.02)	0.75±0.2	1.63±0.8	(P<0.01)

NS = Non significant.

DISCUSSION

Results of this study revealed that there was a significant decrease in α globulin in animals suffering from inactive ovaries versus cycling animals. Meanwhile total protein , albumin % and B globulin were non significantly reduced in cases of ovarian inactivity. El-Azab et al., (1988) discussed the possible causes of ovarian inactivity which associated with lower serum protein and attributed these to hormonal imbalance, particularly, gonadotrophins (FSH and LH) which are proteinous in nature.These results also agreed with those reported by Rowlands and Pocock (1976); El-Baghdady, (1979) and Abdel-Rehim (1982) who attributed these changes to insufficient proper diet, poor absorption of dietary constituents from intestinal tract, environmental, seasonal variation as well as general basal metabolic rate (Emara et al., 1989).

On the other hand, Mikhail (1974) reported that cattle with low fertility rate had higher level of serum protein. Dhoble and Gupta (1981) observed similar results in a study on Indian buffaloes and attributed the hyperproteinemia to the increase in globulin fraction, while Hassan et al. (1991) attributed the hyperproteinemia in buffaloes suffering from ovarian inactivity to the retention of nitrogenous substances in the tissues.

Gamma globulin increased significantly in animals suffering from ovarian inactivity (40.48±6.21% vs 25.87±2.66%). This could be attributed to the absence of suppressive effect of progesterone which down-regulates the immune function (Segerson, 1988; Liu and Hansen,1993). Fredrikson et al. (1988) reported that the supposed beneficial effects of increased estradiol on the immune function are probably the result of a lack of detrimental effects being produced by progesterone secreted by CL. Furthermore, low estradiol values in cases of ovarian inactivity may be less detrimental to immune function than are high progesterone values associated with CL function. Moreover, Ramadan et al., (1997) indicated that cyclic changes in progesterone concentrations are the major determinant of immune function and that reduced progesterone concentrations permit estrogens to up regulate uterine immune function.

In the present study, the significant decrease observed in A/G ratio in animals suffering from ovarian inactivity is in agreement with the findings of El-Azab et al. (1988) and Ebtihal (1992) in buffaloes.

Total protein and albumin levels significantly increased during the follicular phase of the oestrus cycle. This increase could be attributed to the oestrus signs in the animals which need

certain amount of optimal protein level (Dutta et al., 1988).

In conclusion, determination of some serum biochemical parameters such as serum proteins could be of value in prediction of some infertility problems in the buffaloes.

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