

observed binding of ^3H α -tocopherol to its hepatic binding protein, presumably through competition from endogenous tocopherol¹¹. Thereafter animals fed the vitamin E-deficient diet were used to establish optimal assay conditions and properties of tocopherol binding protein⁷. In the present experiment, an experimental group fed this diet was also included as a basis for comparing binding activity with different dietary treatments.

The table illustrates that selenium deficiency produced no effect on the specific activity of α -tocopherol binding protein whether activity was calculated as pmoles/mg protein or as pmoles/g tissue. The only significant difference observed was in the content (pmoles/g liver) of binding protein in the liver of animals fed the casein diet. This higher activity probably results solely from an increase in total soluble liver protein concentration (12.9 mg/ml vs 7.8 and 7.2 mg/ml) rather than an effect of selenium. It should be noted that the torula yeast diet is considerably lower in protein (15%) than the casein diet (22%).

These data suggest that either the binding protein is not a seleno-protein or selenium, if present, is not necessary for optimal binding. We cannot eliminate, however, the possibility that because of the low estimated concentration of the binding protein in liver (approximately 200 pmoles/g)⁷, traces of selenium could maintain activity. In deficiency produced by a similar diet, liver selenium levels of 70 ng/g (800 pmoles/g) have been reported^{3,12} representing more than a sufficient amount of selenium on a molar basis to serve functionally in the binding protein. It does, however, seem unlikely that depletion of selenium from normal

levels of 750 ng/g to 70 ng/g (Burk et al.³), over a 10fold decrease, would be entirely without effect on the binding protein, were it a seleno-protein.

Although the function of the hepatic α -tocopherol binding protein is at present unknown, it appears that its binding properties are unrelated to the selenium status of the animal. This system therefore seems unattractive for further study of a functional interaction between selenium and tocopherol.

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Alpha-globulin follicular fluid proteins within small and large bovine follicles¹

E. C. Segerson, Jr, and R. C. Gray

Department of Animal Science, North Carolina Agricultural and Technical State University, Greensboro (North Carolina 27411, USA), 12 December 1977

Summary. Fluids from small (FF-S) and large (FF-L) bovine ovarian follicles were observed electrophoretically for differences in the alpha-globulin protein profile. FF-L possessed a fast migrating alpha-globulin 3 and a greater percentage of FF-L samples contained a higher number of alpha-globulin bands.

Protein components of bovine ovarian follicular fluids (FF) have been investigated mainly to determine whether the proteins resulted from filtration of the blood or were products of granulosa and theca cells^{2,3}. In these studies the fluids aspirated from all follicles were pooled for the determinations and no attempt was made to investigate the possible qualitative protein differences in fluids from follicles of varying sizes. The electrophoretic examination of porcine FF indicated differences in the alpha-globulin profile between fluids from small and large follicles⁴. The intrafollicular role of the alpha-globulins is unknown, but in the pig these proteins may control the development of follicular oocytes through steroid transporting function or by acting as inductor or repressor substances⁴. The purpose of this study was to investigate the electrophoretic properties of the alpha-globulin proteins within bovine follicular fluids of individual small and large intact follicles.

Materials and methods. Blood and ovaries from 18 cows were obtained at a local abattoir. The ovaries (each pair bearing a corpus luteum-CL) were placed in iced physiological saline, and within 3 h of slaughter the diameters of the ovarian follicles were obtained. An arbitrary scale was used to distinguish between fluids from small follicles (FF-

S; 2.0–5.0 mm) and large follicles (FF-L; 5.1–15.0 mm). Fluids were aspirated from individual follicles from each female and kept separate. Blood samples were centrifuged at 2100×g for 20 min with serum collected and frozen. Protein concentration was quantitated for each serum and FF sample using bovine serum albumin as the standard⁵. Serum progesterone was quantitated by radioimmunoassay⁶ to assess CL-function. The specificity of the antisera to progesterone has been characterized⁷.

A 'freeze-thaw' and centrifugation procedure was used to remove fibrinogen from each serum and FF sample⁸. Protein quantitation was repeated and 250 µg of protein from each serum, FF-S and FF-L sample were subjected to polyacrylamide gel-electrophoresis⁶. Migration was toward the anode using Tris-borate buffer (0.03 M; pH 9.0). Gels were stained with 0.2% Coomassie blue G and destained in methanol:H₂O:acetic acid (45:45:10 v/v). The number of stained alpha-globulin bands and the mobility⁹ of these bands plus the albumin band was recorded for each serum, FF-S and FF-L sample. Protein (mg/ml) and mobility data for serum FF-S and FF-L were analyzed by the Newman-Keuls test and differences in the percentage as related to number of alpha-globulin bands among treatments were analyzed by Chi-square¹⁰.

Table 1. Electrophoretic mobility of albumin and alpha-globulin proteins within serum and fluids from small (FF-S) and large (FF-L) follicles.

Item	Albumin	Alpha-globulin band number				
		1	2	3	4	5
Serum (18) ^a	0.887 ± 0.004 ^b	0.799 ± 0.004	0.726 ± 0.004	0.676 ± 0.005 ^c	0.656 ± 0.019	–
FF-S (62)	0.892 ± 0.003	0.803 ± 0.002	0.728 ± 0.002	0.671 ± 0.002 ^c	0.657 ± 0.005	0.639 ± 0.002
FF-L (66)	0.892 ± 0.002	0.803 ± 0.002	0.733 ± 0.002	0.687 ± 0.003 ^d	0.656 ± 0.004	0.639 ± 0.002

^aDenotes total number of samples observed electrophoretically – all samples did not possess the entire profile of the alpha-globulin proteins; ^bmean value ± SE; ^{c,d}mean values within a column with no superscript in common are significant ($p < 0.01$) different.

Results and discussion. Mean protein concentration (including fibrinogen) for serum ($n = 18$), FF-S ($n = 62$) and FF-L ($n = 66$) was 84.2, 63.1 and 66.9 mg/ml, respectively. Serum protein was greater ($p < 0.05$) than FF protein, which agrees with previous reports for the bovine^{2,3}. Electrophoretic mobility data are presented in table 1. Mobility of albumin in serum was not faster than in FF, as suggested in a previous report employing paper electrophoresis². Alpha-globulin 3 migrated faster ($p < 0.01$) in FF-L than in serum or FF-S.

As shown in table 2, the percent of serum, FF-S and FF-L samples possessing more than 3 alpha-globulin bands was 16.7, 29.0 and 51.5%, respectively. This percent value for FF-L was greater ($p < 0.05$) than values for serum and FF-S, suggesting some protein specificity for FF-L. A fast alpha-globulin 3 and a greater percent of alpha-globulin bands in FF-L as compared to FF-S has not been previously reported. Mean serum progesterone concentration

(8.3 ng/ml; range of 0.9 to 12.7 ng/ml) indicated that all but 2 pairs of ovaries contained a functional CL. It appeared that the CL exerted no influence on either albumin or alpha-globulin mobilities or numbers between ipsilateral and contralateral ovaries within FF-S and FF-L groups. In pig FF obtained from pooled FF-S and pooled FF-L samples, FF-S consistently possessed 3 alpha-globulins while FF-L had 4. Mobility differences in 2 alpha-globulins were observed between FF-S and FF-L⁴.

Although little is known of the nature and activity of alpha-globulin FF proteins, activities of specific human serum alpha-globulins have been noted, i.e. antiplasmin, antitrypsin, thyroxine binding, cholinesterase and antichymotrypsin activity¹¹. The possibility that specific differences in the alpha-globulin profile of bovine FF-S and FF-L are related to oocyte maturation, the ovulation process, or both certainly must be considered.

Table 2. Number and percent of samples of bovine serum and fluids from small (FF-S) and large (FF-L) follicles possessing varying numbers of alpha-globulin bands

Item		Number of alpha-globulin bands/sample		
		< 3	3	> 3
Serum (18)	No. ^a	3	12	3
	% ^b	16.7 ^c	66.7 ^c	16.7 ^c
FF-S (62)	No.	4	40	18
	%	6.5 ^d	64.5 ^c	29.0 ^c
FF-L (66)	No.	3	29	34
	%	4.6 ^d	43.9 ^d	51.5 ^d

^a Number of samples containing the designated number of bands; ^b percent of samples containing the designated number of bands; numbers in parentheses denote total number of tested samples; ^{c,d} percent values within columns with no superscript in common are significantly different ($p < 0.05$).

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Muscle specific structural differences in piscine muscle glycogens

R. V. Krishnamoorthy^{1,2} and B. Jamila Begum

Department of Zoology, University of Agril. Sciences, GKVK Campus, Bangalore North-560065 (India), 16 November 1977

Summary. Piscine red muscles are rich in glycogen content. Structurally white muscle glycogens have one glucosidal unit more in their external branches than the red muscles.

It is widely known that distinct molecular species of glycogen exhibiting different susceptibilities to glycogen breakdown may occur in a given muscle³. In order to examine this in case of fish, wherein the concentration of muscle glycogen has been recognized as an aspect of functional specialization⁴, the present investigation has been undertaken.

The red and white muscles of the lateral line musculature and the heart muscles were excised in bulk, pooling the

Table 1. Glycogen levels in the piscine muscles

Fish	mg glycogen/kg wet weight		Cardiac muscles
	White muscles	Red muscles	
<i>Catla catla</i>	586 ± 42	218 ± 36	198 ± 21
<i>Clarius batrachus</i>	546 ± 34	198 ± 28	189 ± 19

Values are $\bar{x} \pm SD$ of 5 observations.