Seminal plasma androgen-binding protein activity in turkeys with normal white or abnormal yellow semen

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Summary. Dihydrotestosterone (DHT) binding activity of normal white and abnormal yellow turkey semen was quantitated by disc-gel electrophoresis in the presence of [^3H]DHT. White seminal plasma had three peaks of activity ($R_f = 0.3, 0.5$ and 0.8). Yellow seminal plasma had a greater protein concentration and [^3H]DHT binding activity averaging 32.5 ± 7.93 pmol DHT/ml compared with 1.45 ± 0.3 pmol DHT/ml for white seminal plasma. The majority of [^3H]DHT binding was localized at $R_f = 0.5$ for the yellow seminal plasma. When labelled samples were separated by electrophoresis on unlabelled gels, the only peak of activity was at $R_f = 0.5$. Blood serum contained 3 peaks of activity ($R_f = 0.4, 0.5$, and 0.8). We conclude that a seminal plasma androgen-binding protein is present in the domestic turkey, and in males with yellow semen syndrome androgen-binding activity is increased.

Introduction

Androgen-binding protein (ABP) is a specific androgen-transporting protein (Tindall & Means, 1980) which has been isolated from the male reproductive system of several mammals. It is produced primarily by Sertoli cells (Hagenas *et al.*, 1975; Lipshultz, Murthy & Tindall, 1982), has a high affinity for 5α -dihydrotestosterone (DHT; Lobl, 1981) and differs from sex steroid binding protein which is found in blood serum (Tindall & Means, 1980; Lobl, 1981).

ABP is normally recovered and characterized from extracts of testes or epididymides but it has been found also in seminal plasma (Jegou, Dacheux & Terqui, 1978; Jegou & Le Gac-Jegou, 1978; Plymate, Fariss, Smith, Jacob & Matej, 1981; Oda, 1982). Its purported function is the transport of androgen to the epididymis (French & Ritzén, 1973; Danzo, Cooper & Orgebin-Crist, 1977) where it is taken up by epithelial cells (Tindall & Means, 1980; Pelliniemi *et al.*, 1981). Proper function of the ductal cells may be dependent on androgen delivered by this mechanism (Hamilton, 1971).

The domestic turkey normally produces white coloured semen, but there is an endemic abnormality known as the yellow semen syndrome which is associated with reduced fecundity. The yellow discoloration is confined to the seminal plasma which also has elevated concentrations of protein, cholesterol and DHT (Thurston, Hess, Biellier, Addinger & Solorzano, 1975; Hess & Thurston, 1982; Hess, Thurston & Biellier, 1982; Thurston, Hess, Froman & Biellier, 1982). Other sequelae include abnormal spermatids, numerous macrophages and steatosis of the epithelia of the ductuli efferentes (Thurston *et al.*, 1982). The pathology of yellow semen syndrome, particularly of the epididymal region, could be a manifestation of abnormal metabolism or transport of androgen. The presence of ABP or its binding activity has not been demonstrated in avian semen or

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reproductive tissues. We have therefore measured the binding activity for androgens in the seminal plasma of normal turkeys and compared the activity to that in semen from males with yellow semen.

Materials and Methods

The DHT-binding activity of seminal plasma proteins was determined by disc-gel electrophoresis with tritiated [^3H]DHT and steady-state equilibrium conditions (Ritzén, French, Weddington, Nayfeh & Hansson, 1974). Turkey blood serum was included for comparison of protein bands and binding activity. Rat epididymides served to validate the assay.

Methods. Protein concentrations were determined for all samples using a Coomassie Brilliant Blue G250 assay (Bio-Rad Laboratories) with purified bovine serum albumin as a standard. Each standard curve was analysed by a least squares 'lack of fit' method.

Polyacrylamide gels were prepared according to Davis (1964) and the procedure for electrophoresis was similar to that described by Ritzén *et al.* (1974). Each gel contained two layers of 6.5% acrylamide, 0.1% *N,N'*-methylene-bis-acrylamide and 10% glycerol in 0.1 M-Tris-HCl buffer (pH 8.9). The bottom layer was 60 $\times$ 5 mm and contained 2 nM-5 α -[1,2,4,5,6,7,16,17- ^3H]dihydrotestosterone; and the top layer was 6 $\times$ 5 mm and contained 10 nM-[^3H]DHT. Both layers were polymerized at the same time. Gels for non-specific binding contained 2 nM-[^3H]DHT and 400 nM unlabelled DHT in both layers. Gels for indicating DHT affinity contained no steroid, but the samples were incubated with 4 nM-[^3H]DHT. The [^3H]DHT was added to the gel solutions from a stock preparation containing 5 $\mu\text{Ci}/100\ \mu\text{l}$ ethanol. Gels were run for approximately 3 h at 1 mA per tube with 4.5 mM-Tris-glycine electrode buffer (pH 8.3) at 4°C. After electrophoresis, the gels were cut into 2.4 mm slices, incubated overnight at room temperature in 5 ml toluene:liquifluor (24:1, v/v: New England Nuclear), and counted for 5 min or to 2% accuracy at 58.5% efficiency. Other gels were stained in Coomassie Brilliant Blue G250 for elucidation of the banding pattern. Calculations were made according to Ritzén *et al.* (1974) for steady-state binding activity.

Experiment 1. Three groups of 10 males each were selected on the basis of seminal plasma protein concentration and colour (Thurston *et al.*, 1975) from a flock of 500 commercial breeders. Groups were as follows: (1) low protein (3.2–6.0 mg/ml) and white colour; (2) medium protein (6.1–18.2 mg/ml and white colour; and (3) high protein (23.0–51.4 mg/ml) and yellow colour. Semen was collected 3 times from all males with a minimum intercollection period of 2 days. The semen was centrifuged at 13 000 *g* for 5 min and the supernatant seminal plasma was stored at –40°C. For analysis of blood serum, blood was withdrawn from males in Groups 2 and 3 at 2-h intervals before semen collection and the serum was pooled.

Epididymal extracts from 5 adult rats were also analysed. Extracts were obtained by homogenization of tissues in 0.01 M-Tris-HCl buffer (3:1 buffer:tissue; 10% glycerol; pH 7.4) at 4°C using a polytron (Brinkmann Instruments), then centrifuged at 10 000 *g* for 15 min. The supernatant was further ultracentrifuged at 100 000 *g* for 1 h, then stored at –40°C before analysis.

Samples were thawed at 4°C and protein concentration was determined. Blood and semen samples were diluted with the Tris-HCl buffer (pH 7.4) to a protein concentration of 5 mg/ml. Endogenous steroids were removed from all samples by addition of charcoal (acid-washed, oven-dried, Norit A; 1 mg/mg protein). After 10 min the charcoal was separated from the supernatant by centrifugation.

The supernatants of all samples were made up to 4 nM-[^3H]DHT, 20% glycerol and 0.001% bromophenol blue. Samples for determination of non-specific binding were diluted with buffer containing 800 nM unlabelled DHT, in addition to 8 nM-[^3H]DHT. Relative affinity for DHT was assessed in semen samples from Groups 2 and 3 by adding the labelled seminal plasma to unlabelled gels. Each electrophoretic gel contained 0.3 mg protein.

Experiment 2. Semen was collected once from 5 males with white semen and 8 with yellow semen and the plasma was stored at -40°C . Individual samples were evaluated for ABP activity as for Exp. 1.

Statistical analysis. The results of Exps 1 and 2 were analysed separately because of the differences in sampling, i.e. pooled versus individual. Group variances for binding activity were not homogeneous amongst protein groups (computed as the ratio of their variances); therefore, multiple comparisons among means (Exp. 1) were made after $\log_e$ transformation. Comparisons between two means (Exp. 2) were made by the Student's t distribution with different variances (Ott, 1977).

Results

DHT binding activity was found in pooled and individual turkey seminal plasma, having an electrophoretic mobility (R_f) value of approximately 0.3, 0.5 or 0.8 (Text-figs 1a & 1c). Activity at $R_f = 0.3$ or 0.8 was more frequently detected in normal white seminal plasma, whereas yellow seminal plasma binding activity was especially pronounced at $R_f = 0.5$. The increased binding activity at $R_f 0.5$ in yellow semen corresponded to a particular band as determined by Coomassie Blue stain (Text-fig. 1c), but was less discernible in white semen (Text-fig. 1a). Significant radioactivity was detected at the beginning of the gels in all samples ($R_f = 0.17$), but was usually more prominent in samples with the least DHT-binding activity. Therefore, this was presumed to be residual radioactivity from the 10 nM-[^3H]DHT in the top layer of the gels.

Activity was particularly high in yellow seminal plasma (Tables 1 & 2), and the increase in specific activity as seminal plasma protein concentration increased was disproportionate. This indicates that the protein responsible for binding at $R_f = 0.5$ may have increased in concentration at a greater rate than for other plasma proteins in yellow semen. Binding activities for the pooled samples are reported as geometric means (Table 1). Individual yellow seminal plasma samples had a large variation in activity (Table 2), but, as for the pooled samples, bound more [^3H]DHT than did seminal plasma from normal males.

Text-fig. 1. Steady-state polyacrylamide gel electrophoresis of seminal plasma (a, c) and blood serum (b, d) from turkeys with white (a, b) or yellow (c, d) semen (Exp. 2). Stained gels under each plot are aligned according to protein migration, from cathode (–) to anode (+). BP = bromophenol blue front. R_f values are indicated above the plots.

Table 1. Protein concentration and androgen-binding activity in turkey seminal plasma (Exp. 1)

Group	Semen colour	No. of reps	Protein* (mg/ml)	Binding activity*	
				pmol DHT/ml	pmol DHT/mg protein
1	White	3	6.81 ± 0.04 ^a	0.413 ± 0.106 ^a	0.076 ± 0.030 ^a
2	White	3	11.15 ± 0.04 ^b	1.408 ± 0.106 ^a	0.150 ± 0.030 ^b
3	Yellow	3	41.45 ± 0.04 ^c	22.155 ± 0.106 ^b	0.607 ± 0.030 ^c

* Pooled seminal plasma from 10 males.

* Geometric means ± s.e. Different superscripts within a column indicate a significant difference ($P \leq 0.05$).

Table 2. Protein concentration and androgen-binding activity in turkey seminal plasma (Exp. 2)

Semen colour	No. of samples	Protein* (mg/ml)	Binding activity*	
			pmol DHT/ml	pmol DHT/mg protein
White	5	9.5 ± 0.36 ^a	1.45 ± 0.30 ^a	0.153 ± 0.030 ^a
Yellow	8	35.85 ± 4.04 ^b	32.50 ± 7.93 ^b	0.850 ± 0.115 ^b

* Means ± s.e. Different superscripts indicate a significant difference ($P \leq 0.05$).

Text-fig. 2. Radioactivity in gel slices from tritiated samples of white and yellow seminal plasma which was electrophoresed on an unlabelled gel (a). (b) Non-specific binding as determined by steady-state polyacrylamide gel electrophoresis with gels containing excess unlabelled DHT (400 nM) in addition to 2 nM-[³H]DHT. BP = bromophenol blue front. The R_f value of 0.5 is indicated.

Seminal plasma from birds with white and yellow semen incubated with [³H]DHT but electrophoresed with gels containing no steroid, still showed binding activity with an $R_f = 0.5$ (Text-fig. 2a). Non-specific binding was insignificant as determined by gels containing 2 nM-[³H]DHT and an excess of unlabelled DHT (Text-fig. 2b).

DHT-binding activity was found in at least three peaks in blood serum samples from a normal male with white semen; R_f values were about 0.4, 0.5 and 0.8 (Text-fig. 1b). Serum from a male with yellow semen showed more binding at 0.4 than at 0.5 and no activity at 0.8 (Text-fig. 1d). Blood serum and seminal plasma albumin had little binding activity and migrated with an R_f of approximately 0.68 and 0.64, respectively (Text-figs 1a-d).

Rat epididymis contained a distinct binding activity (977.3 ± 115.9 fmol DHT/mg protein) with an R_f of 0.39, similar to that found by others (Tindall *et al.*, 1975; Lipshultz *et al.*, 1982).

Discussion

The seminal plasma DHT-binding activity at an approximate R_f of 0.5 was more than 20 times higher in yellow turkey semen than in normal white semen, and may represent a specific seminal plasma androgen-binding protein. The association of reproductive problems with an increase in ABP activity has not been reported (Lobl, 1981); however, a decreased activity of ABP has been associated with genetic reproductive failure in male rats (Musto & Bardin, 1976). Due to insufficient semen volume, the relative binding affinity and specificity were not determined for the DHT-binding in the present study.

ABP with an R_f of 0.5 has been detected in mammalian semen (Jegou *et al.*, 1978) and in testicular and epididymal extracts (Sanborn, Elkington, Tcholakian & Steinberger, 1975; Jegou *et al.*, 1978; Carreau, Drosdowsky & Courot, 1979; Feldman *et al.*, 1981). In other reports, seminal ABP was found to migrate near $R_f = 0.3$ (Jegou & Le Gac-Jegou, 1978; Barahona, Banuelos, Solis & Bermudez, 1980; Plymate *et al.*, 1981), which is similar to one peak of activity in the white turkey seminal plasma. Since yellow semen contained much more activity at R_f 0.5, the protein responsible may have removed the [^3H]DHT from the slower moving peaks, indicating that binding at R_f 0.3 or 0.8 was of low affinity.

Other possible explanations of DHT-binding in turkey semen include blood plasma steroid-binding proteins and cytoplasmic androgen receptors. Corticosterone binding globulin (CBG), an α -globulin (Daughaday, 1959), migrates with an R_f near 0.5 and binds testosterone in addition to cortisol and progesterone (Corvol, Chrambach, Rodbard & Bardin, 1971; Martin & Ozon, 1975; Wagner, 1978; Barahona *et al.*, 1980). However, the seminal plasma binding activity is probably not due to CBG, because [^3H]DHT peaks do not appear in PAGE or agar electrophoresis of human blood plasma (Wagner, 1978; Barahona *et al.*, 1980).

Sex steroid-binding protein (SBP), in contrast, binds DHT and testosterone with high affinity (Wagner, 1978). SBP is a β -globulin, and SBP from several mammals migrates with an R_f of about 0.3 (Corvol *et al.*, 1971; Purvis, Calandra, Sander & Hansson, 1978; Barahona *et al.*, 1980). However, SBP has not been detected in adult avian blood (Corvol & Bardin, 1973; Salhanick & Callard, 1979), although it has been demonstrated in turtles, fish and amphibians (Salhanick & Callard, 1979).

The present study suggests that steroid-binding proteins of undetermined specificity exist in turkey blood with peaks of activity at $R_f = 0.4$ and 0.5. The activity at $R_f = 0.5$ was decreased in blood from a male with yellow semen while that at $R_f = 0.4$ was increased. It is therefore possible that the increased activity at $R_f = 0.5$ in yellow semen may be due to influx of a blood plasma DHT-binding protein. Tindall, Cunningham & Means (1978) suggested that SBP may be transported from blood into the reproductive tract because, in the rabbit, SBP decreased and ABP increased in concentration with age.

The elevated levels of protein in yellow semen appear to originate from excessive apocrine secretion and cytoplasmic extrusion by non-ciliated cells of the ductuli efferentes (Hess & Thurston, 1977; Hess *et al.*, 1982). With cellular damage or excessive blebbing, androgen receptors from the cytoplasm may be released into the ductal lumen. However, cytoplasmic receptors are not thought to be responsible for the increased DHT-binding activity in yellow semen. The dissociation of DHT from cytosol receptors requires days rather than minutes (Tindall & Means, 1980). In the present study, the amount of [^3H]DHT bound to turkey seminal plasma ABP was greatly reduced after electrophoresis for 3 h in unlabelled gels.

It is possible that the binding protein is synthesized by epithelial cells of the epididymal region and that, in the yellow semen syndrome, the extrusion of excessive amounts of cytoplasm includes a DHT-binding protein. Alternatively, a high-affinity ABP may serve as a reservoir that binds excess androgen in the ductal lumen. Males with the yellow semen syndrome have high levels of seminal plasma DHT (Hess, 1983). The increased binding activity concomitant with an increase in an ABP may be consequential to elevated DHT.

Published with approval of the Director of the South Carolina Agricultural Experiment Station as Technical Contribution No. 2188.

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Received 5 September 1983