Chapter – V

Role of male Mullerian gland of caecilians (Amphibia: Gymnophiona) in secretions of seminal plasma: a study in *Uraeotyphlus narayani* adopting biochemical and immunological tools
INTRODUCTION

The caecilians (Amphibia: Gymnophiona) comprise a unique group of subterranean limbless amphibians with discontinuous distribution in the tropical countries (Nussbaum and Wilkinson, 1989). Whereas the other group of amphibians, with rare exceptions, have an aquatic phase in the life cycle when the eggs are laid and the young ones hatch out in water, several species of caecilians are viviparous and totally skip the aquatic phase in the life cycle. Between the total dependence on water for completion of the life cycle and the one skipping an aquatic phase, there are different grades (Wilkinson and Nussbaum, 1998). The uniqueness of the ecological, morphological, anatomical, physiological and embryological features of the caecilians have been inferred to indicate an early attempt in evolution towards terrestrialization (Wake, 1970). This is supported by the internal fertilization, making use of an eversible phalodeum as the intromittent organ or phallus (Wake, 1977; Himstedt, 1996; Gower and Wilkinson, 2002).

Among the vertebrates, internal fertilization is characteristic of the amniotes. In the amniotes the sperm are suspended in a medium which is the product of secretion of one or more glands. In the reptiles contributions to seminal plasma come from the epididymis (Depeiges and Dufaure, 1977; Shahul Hamid and Akbarsha, 1989; Manimekalai and Akbarsha, 1992; Velmurugan and Akbarsha, 1992; Akbarsha and Manimekalai, 1999), ampulla ductus deferentis (Akbarsha and Meeran, 1995; Daisy et al., 2000) and the renal sex segment (Bishop, 1959; Sarkar and Shivanandappa, 1989). In the birds, the epididymis, ductus deferens and receptacle together produce...
secretions which comprise the main component of the seminal plasma and act as fluid vehicle for the sperm and provide a medium in which sperm mature and develop the potential for motility (Birkhead, 1998). In the mammals, particularly the placentals, about 30% of the seminal plasma is the contribution from the prostate gland, 60% from the seminal vesicles and the rest from the testis, epididymis, ampulla ductus deferentis and bulbourethral gland (Lilja et al., 1989; Aumuller and Seitz, 1990; Setchell et al., 1994; Luke and Coffey, 1994). The secretory material from the various male accessory reproductive glands of mammals contain fluid, proteins, fructose, spermine, citric acid, sialic acid, enzymes, prostaglandins, lipids, zinc, etc., which contribute to the sustenance of sperm motility; some of the constituents inhibit sperm motility and/or delay the capacitation of the spermatozoa (Luke and Coffey, 1994; de Lamirande et al., 2001).

In the caecilian stage of evolution, male accessory reproductive glands comparable to epididymis, renal sex segment, ampulla ductus deferentis, prostate gland, seminal vesicles and bulbourethral gland are absent. Yet, the caecilians practice internal fertilization (Spengal, 1876; Tonutti, 1931; Wake, 1977a, 1977b; Himstedt, 1996; Gower and Wilkinson, 2002). The organization of the male urinogenital system of the caecilians is, by and large, similar to that of the other amphibian groups. Spermatozoa, produced in the extended lobes of the testis, pass through the collecting ductules into the kidney from where they take the course of the urine to reach the cloaca (Wake, 1970; Smita et al., 2002). Yet, the fact remains that internal fertilization requires a medium in which sperm are suspended during ejaculation. The male caecilians possess an unique gland known as Mullerian gland which is a derivative of the embryonic Mullerian duct (Wake, 1981). In other words the Mullerian duct, which should disappear in the male caecilians under the influence of anti-Mullerian hormone (AMH), is retained in the adult males as a functional gland (Wake, 1981).

Investigators have attributed the role of male accessory gland to the Mullerian gland of caecilians. This is supported by the fact that the prostate gland of mammals develops from the anterior urogenital sinus, which is a diverticulum of the degenerating Mullerian duct (Narbaitz, 1974; Wake, 1981; Luke and Coffey, 1994). The pioneering histological and histochemical investigations of Wake (1977a, 1977b,
1981) have shown that the caecilian Mullerian gland secretion is in the form of granules which are rich in mucopolysaccharides, monosaccharides viz., glucose and fructose, lipids and acid phosphatase. Exbrayat (1985), in his scanning electron microscopic study, has provided additional proof for secretion of discrete granules from the Mullerian gland of *Typhlonectes compressicauda*. Whereas proteins are a major constituent of the secretory material of all the male accessory reproductive glands of amniotes referred *vide supra*, according to Wake (1981), some proteins (positive Millon reaction for tyrosine which occurs in most proteins) is present in the secretory cell of the Mullerian gland of *Dermophis mexicanus* and *T. compressicauda* but it may be a property of cell maintenance rather than secretion. However, Exbrayat (1985), in his work on *T. compressicauda*, reported two kinds of secretory granules, one which is not PAS\(^{+}\) but is metachromatic and stained by alcian blue at pH 2.5, indicating it to be of protein nature and sometimes acidic glucidic part, and the other is PAS\(^{+}\) but neither metachromatic nor stained by alcian blue indicating it to be of neutral glycoprotein nature. Thus, to date there has been no attempt to subject the proteins of caecilian Mullerian gland to electrophoretic analysis so as to characterize them and to infer on their source and destination. The present study in *Uraeotyphlus narayani* fills this gap and reports five of the proteins of the Mullerian gland to be glycoproteins. An attempt was also made adopting immunodiffusion, immunoelectrophoresis and immunoblot analyses, to find the antigenic similarity of the proteins of the Mullerian gland, testis, liver and blood of this caecilian. The paper also reports occurrence of acid phosphatase and Ca\(^{2+}\) and Na\(^{+}\)-K\(^{+}\) ATPases in the Mullerian gland. Further the paper confirms the presence of a monosaccharide in the secretory material of the Mullerian gland, which Wake (1981) in *D. mexicanus* and *T. compressicauda* demonstrated adopting spot tests.

**MATERIALS AND METHODS**

**Animal collection and maintenance**

*Uraeotyphlus narayani*, weighing approximately 22-26g, and measuring a length of 20-27cm, were chosen for the study. Ten male animals were collected from the coconut groves and rubber plantations in an around Thodupuzha in Idukki District,
Kerala, India (Lat 09° 53′ 52″ N Long 76° 42′ 29″ E), during different months of active phase (July to February) and three animals during the period of testicular quiescence (March to June). In the laboratory the animals were kept alive in moist soil and fed on live earthworm.

**Preparation of samples**

The animals were anesthetized using MS222. Blood was collected directly from the heart and the serum was separated. After collecting the blood, the animals were dissected to expose the viscera. Tissue samples of Mullerian gland, testis, and liver were washed thoroughly in amphibian physiological saline solution, pH 7.4 (Dubin and Dionne, 1994) (NaCl 130mM, HEPES 10mM, glucose 5mM, pyruvic acid 5mM, CaCl₂ 3mM, KCl 2.5mM, MgCl₂ 1mM) and homogenised in Tris-HCl buffer (pH 7.2) using an all glass homogeniser. The homogenates were centrifuged at 10,000 x g for 20 min and the supernatant was separated for further analysis. The protein content of the extracts of Mullerian gland, testis, liver and serum were determined spectrophotometrically adopting the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

**SDS-PAGE analysis**

Polyacrylamide gel electrophoresis (PAGE) was conducted according to Laemmli (1970). 50μg of the protein samples were loaded on 12% gel. High as well as low MW marker proteins (SIGMA molecular weight markers for SDS-PAGE) were run simultaneously for comparison. Electrophoresis was carried out at 50V for 8 hr. Gels were stained with Coomassie brilliant blue (Laemmli, 1970) or periodic acid-Schiff’s reagent (Fairbanks et al., 1971, modified by Flickinger et al., 1986). An estimate of the total expressed Mullerian gland proteins was determined using Alpha image Gel Documentation and analysis (Alpha Innotech Corporation, USA).

**Immunoassays**

Qualitative immunodiffusion was done following the method of Ouchterlony (1949). Antiserum to Mullerian gland proteins was produced in the rabbit against the
protein precipitated using ammonium sulphate (Scopes, 1994). Immunodiffusion plates were pre-coated using agarose. The central well in the immunodiffusion plate was filled with 10 μl of antiserum and the peripheral wells were filled with 10 μl each of various antigens that were to be tested. The plates were incubated inside a humid chamber for 72 hr. The precipitin arcs were stained with 0.025% Coomassie brilliant blue.

Qualitative immunoelectrophoresis was carried out following the method of Feinstein (1976). Precoated microslides were used for preparation of plates. Two antigen wells and an antiserum trough were punched. The wells were filled each with 5 μl of antigen samples mixed with marker dye and electrophoresis was carried. At the end the plates were taken out and the central agar strip was removed from the antiserum troughs. The trough was filled with antiserum and the plates were incubated in a humid chamber for 48 hr. The precipitin arcs were stained with 0.025% Coomassie brilliant blue.

Immunoblot analysis was carried out according to the method of Towbin et al. (1979). Immediately after the electrophoretic separation of the proteins, the gels were washed in glass distilled water and soaked in transfer buffer for 30 min. Nitrocellulose (NC paper) and gel were soaked in transfer buffer and the transfer was carried out in the trans-blot apparatus (BIO-RAD, USA). After transfer, the NC paper was dried, and incubated in blocking solution for 3 hr with gentle shaking. The NC paper was incubated with the polyclonal primary antibody in 1:1000 dilution for 3 hr. The NC paper was then washed. The primary antibody was probed with the secondary antibody (anti-rabbit IgG). Incubation was carried out for 3 hr and the NC paper was allowed to develop color by the addition of benzidine as the substrate. The reddish brown-coloured bands were photographed.

**Enzyme assays**

**Qualitative analysis of Acid phosphatase**

The method used in preparation of 8% native gel was that of Vallejos (1983). Electrophoresis of the protein extract of Mullerian gland was carried out. The staining
solution consisted of 1% fast blue was dissolved in acetate buffer (pH 5.5) and 0.5% α-naphthylphosphate was added. The gel was incubated in dark at 30°C for 1 to 5 hr or until coloured band appeared.

Determination of acid phosphatase activity of Mullerian gland and serum

The method used in this assay was according to Tenniswood et al. (1976). The level of protein in the sample was determined according to Bradford (1976). 0.5 ml of 4% p-nitrophenyl phosphate and 0.5 ml of 0.1 M citrate buffer (pH 4.8) were pipetted out into clean glass tubes. The tubes were placed in water bath for 5 min at 37°C. The reaction was then initiated by the addition 0.1 ml of tissue or serum extract. After exactly 30 min incubation, the reaction was arrested by the addition of 3.8 ml of 0.1 N NaOH. The reaction product, p-nitrophenol, was measured spectrophotometrically at 415 nm against a reagent blank. The activity level of ACP was obtained using standard curve for p-nitrophenol. The enzyme activity was expressed as nM of p-nitrophenol mg⁻¹ protein h⁻¹.

Adenosine triphosphatase (ATPase)

The total ATPase activity was determined according to the method of Shiosoko et al. (1971). The inorganic phosphorus is estimated adopting the method of Fiske and Subbarow (1925). The aliquots of enzyme source were prepared by extracting the tissue in 0.25 mM sucrose solution, containing 1 mole of EDTA (pH 7.5) and 0.001 M imidazole. The reaction mixture contained 0.2 ml of Tris-HCl buffer (0.1 ml of 100 mM MgCl₂, 0.1 ml 100 mM NaCl, 0.1 ml of 100 mM KCl, 0.1 ml of 100 mM CaCl₂, 0.1 ml of supernatant and 0.1 ml of ATP solution. The contents were incubated at 37°C for 15 min. The reaction was terminated by the addition of 2 ml of 5% TCA. The tubes containing the reaction mixture were kept in the cold (4°C) for 30 min. To the supernatant, 1 ml of ammonium molybdate solution and 0.4 ml of aminonaphthol sulphonic acid reagent (ANSA) were added. The color developed was read at 680 nm against a reagent blank. The protein content was determined according to Lowry et al. (1951). Standard curve for inorganic phosphate was prepared using anhydrous
monopotassium phosphate. The enzyme activity was expressed as μM of inorganic phosphate formed mg⁻¹ protein min⁻¹.

Paper chromatographic analysis of monosaccharides

The presence of sugar in the Mullerian gland was analysed using paper chromatography according to the method of Trevelyan et al. (1966). The sugar from the Mullerian gland was extracted using 5% trichloroacetic acid. The supernatant thus obtained was used for the paper chromatographic separation of sugars. 10μl of the sample was spotted into the chromatographic paper and air-dried. 5% glucose and fructose were used as standards. After spotting of the sample and the standards on to the paper, the paper was placed over the solvent system (isopropanol: water, 8: 2) and allowed to run. After the run, the chromatographic paper was air-dried and dipped in silver nitrate solution, then ethanolic sodium hydroxide and bleached in 6N NH₃ solution. The paper was then extensively washed in H₂O, fixed in sodium thiosulphate and washed in distilled water. The paper was air-dried and photographed.

RESULTS

SDS-PAGE analysis

SDS-PAGE analysis of proteins of the Mullerian gland during the period of active spermatogenesis, stained using Coomassie brilliant blue, revealed a total of 25 fractions. Fraction No.1, the slowest moving fraction, is MW 220 kDa, and the fraction No. 25, the fastest moving fraction, is MW 3 kDa (Fig.1). Fractions with MW 97, 94, 80, 66, 62, 43, 29, 28, 24, 22, 20, 16, 14 and 6 kDa may be considered as the prominent fractions. Fraction with MW 14 kDa appears to be a composite protein, formed of two units, differing slightly in electrophoretic mobilities. The details of distance, width, height, area and percentage of area of the various fractions are in the table 1.
Following is the observation pertaining to the proteins fractions of similar electrophoretic mobilities appearing in the Mullerian gland, testis, liver and serum of *Uraeotyphlus narayani*: Protein with MW 43, 28 and 16 kDa are present in all the four tissues whereas those with MW 18 and 6 kDa appear in testis and liver but not in the serum (Fig. 2).

PAS staining of the gels revealed that out of the 25 fractions of Mullerian gland proteins, MW 43, 28, 24, 14 and 6 kDa proteins are glycoproteins (Fig. 3). Interestingly, fraction with MW 43 kDa alone appears to be common between the Mullerian gland, testis, liver and blood (Fig. 3). Two separate fractions, MW 15 and 13 kDa appear in the liver, blood as well as testis, but are absent in the Mullerian gland, which has a composite 14 kDa fraction. Though quite a few fractions, in terms of MW, appear to be common between three or more tissues, only fraction with MW 43 kDa is definitely common for all the four tissues and in all tissues it is a glycoprotein.

Whereas almost all fractions up to MW 28 kDa appear in the regressed Mullerian gland, all other fractions, which are fast moving ones, are absent in the regressed gland. Even the fractions with MW 66 and 43 kDa, which are prominent in the active gland, show up only as faint bands in the regressed gland (Fig. 4). The data pertaining to the densitometric scanning of the various gels are in the figure 5.

**Immunodiffusion analysis**

The immunodiffusion analysis produced several precipitin lines (Fig. 6a). Precipitin arcs also appeared between antibody against Mullerian gland proteins and proteins of liver (Fig. 6a), testis (Fig. 6b) and serum (Fig. 6c) of *Uraeotyphlus*.

**Immunoelectrophoretic analysis**

The Immunoelectrophoretic analysis produced results comparable to those from immunodiffusion analysis (Figs. 7a-c). Whereas precipitin lines were several
between the Mullerian gland antibody and the proteins of the Mullerian gland, there were only a limited number of precipitin lines appearing between Mullerian gland antibody and proteins of testis (Fig. 7a), liver (Fig. 7b) and serum (Fig. 7c) of *Uraeotyphlus*.

**Immunoblot analysis**

Immunoblot analysis revealed that the polyclonal antibody raised against the proteins of Mullerian gland immunoreacted with all proteins of the Mullerian gland, and proteins of the testis with MW 192, 97, 66, 43, 28, 16 and 14 kDa (Fig. 8). The antibodies also found immunological homogeneity in the liver proteins with MW 192, 66, 28 and 14 kDa, and in the blood with MW 43, 28, 14 (for 14 kDa protein, the fractions in the liver and blood are with MW 13 and 15 kDa).

**Acid phosphatase**

Qualitative analysis of acid phosphatase of *Uraeotyphlus* Mullerian gland, revealed the presence of relatively high molecular weight protein (Fig. 9). Data on the ATPase activity of Mullerian gland, compared to that in the serum, are in the table 2. The activity level in the Mullerian gland is ten times greater than in the serum.

**ATPase of the Mullerian gland**

Analysis of the Ca$^{2+}$ ATPase and Na$^+$-K$^+$ ATPase revealed the occurrence of these ATPases in the Mullerian gland of *Uraeotyphlus*. The activity levels were $1.79 \pm 0.08$ nM of PO$_4^{2-}$/min/mg proteins for Ca$^{2+}$ ATPase, and $0.21 \pm 0.03$ nM of PO$_4^{-}$/min/mg protein for Na$^+$-K$^+$ ATPase.

**Monosaccharide**

The paper chromatographic analysis of the monosaccharides revealed the presence of a sugar corresponding in mobility to both glucose and fructose (Fig. 10).
DISCUSSION

The present work is the first ever attempt to analyze electrophoretically the proteins of the Mullerian gland of any caecilian. Wake (1981) made a pioneering study to find the chemical nature of the secretory material of male Mullerian gland of the caecilians *Dermophis mexicanus* and *Typhlonectus compressicauda* adopting histochemical techniques. She produced evidence for secretion of nonsulphated acid mucopolysaccharides, glycogen, lipids, acid phosphatase, tyrosine-rich proteins, glucose and fructose. Exbrayat (1985) in his histochemical analysis of the Mullerian gland of *Typhlonectus compressicaudus* showed the presence of a PAS⁺ material which is neither heterochromatic nor stained by alcian blue, indicating neutral glycoproteins, at one portion of gland, and a material not ever PAS⁺ but metachromatic and stained by alcian blue at pH 2.5, indicating acidic glycoproteins, at another portion of gland. The present study unambiguously shows that the Mullerian gland of *Uraeotyphlus narayani* secretes several proteins. Out of these, five are glycoproteins and the rest are proteins without carbohydrate moieties. Wake (1981) reported the presence of acid phosphatase in the caecilians she worked with, based on histochemical tests, and the present study reports acid phosphatase on native gel appearing as a single but dense band, and its activity to be about 10 times higher than in the serum. The present study also reports the presence and activity levels of Ca^{2+}-ATPase and Na⁺ K⁺-ATPase. Occurrence of a monosaccharide is also demonstrated. Thus, the present paper substantiates the concept of Tonutti (1931), Wake (1981) and Exbrayat (1985) that the caecilian Mullerian gland is a secretory organ. Our earlier study (Chapter 3) has produced definite evidence for the posterior ends of the ducts of the Mullerian gland and male urinogenital duct of each side in *U. narayani* joining to form a common duct which in turn opens into the cloaca. This finding, together with the present findings and the findings of the Wake (1981) and Exbrayat (1985), establish that the caecilian male Mullerian gland secretion, which is rich in non-glycosylated proteins and glycoproteins, is added on to the sperm before ejaculation. Thus, the present study establishes the caecilian male Mullerian gland as the male accessory reproductive gland (MARG).
As MARG, the caecilian Mullerian gland should be secreting substances for addition to the semen, comparable to those in the amniotic vertebrates, since in both cases i) fertilization is internal, ii) the sperm are suspended in a fluid medium which forms the vehicle of transport, and iii) constituents of the MARG secretions contribute to modulation and energetics of motility and to capacitation of the sperm. Therefore, a comparison of the proteins of the male Mullerian gland of *Uraeotyphlus* with those of the prostate gland of mammals is pertinent, since i) the prostate gland is a derivative of the anterior urinogenital sinus which in turn is a diverticulum of the degenerating Mullerian duct (Narbaitz, 1974; Wake, 1981), and ii) anatomically, in the caecilians, the male Mullerian gland is connected to the spermatic pathway at that part which in the mammals corresponds to the one at which the prostate gland and seminal vesicles are connected (Setchell et al., 1994).

The major proteins contributed to the semen by the prostate gland are i) prostate specific antigen (PSA), also known as seminin or gamma-seminoprotein (\(\chi\)-SM), ii) prostate-specific protein (PSP-94), also known as beta-microseminoprotein (\(\beta\)-MSP) or beta-inhibin, and iii) prostatic acid phosphatase (PAP) (Lilja and Abrahamsson, 1988; Luke and Coffey, 1994). Human PSA is a glycoprotein of 33000 MW and contains 7% carbohydrate (Watt et al., 1986) and there appears to be no homologue of this protein in the other mammals (Kwong et al., 2003). There is no protein in *Uraeotyphlus* Mullerian gland which is a glycoprotein, matching the MW of human PSA. It is understandable because PSA is a tumor-specific antigen, and it has no homologue even in the other mammals (Luke and Coffey, 1994). It is considered to be the sperm motility inhibitor protein (Jeng et al., 2001; Chao et al., 1996).

Prostate specific protein (PSP-94) is a major 16kDa MW protein found in prostatic secretion and contains 94 amino acids (Luke and Coffey, 1994). It is a non-glycosylated and cysteine-rich protein and has a homologue in the rodent (Kwong et al., 2003), rhesus monkey (Nolet et al., 1991; Manaskova et al., 2002) and boar (Manaskova et al., 2002). *Uraeotyphlus* male Mullerian gland has a non-glycosylated protein with MW 16 kDa. Therefore, it will be pertinent to pursue work further to find if this protein of *Uraeotyphlus* is the homologue of mammalian PSP 94, which can
throw light on the evolutionary relationship between caecilian male Mullerian gland and mammalian prostate gland.

In the mammals ACP activity is 200 times greater in the prostate tissue than in any other tissue (Luke and Coffey, 1994). ACP is a family of unrelated enzymes that catalyse the hydrolysis of phosphate monoesters, and in some cases phosphoryl transfer between a phosphodiester and an alcohol. Human prostatic ACP is a glycoprotein dimer of MW 102 kDa, and contains about 7% by weight of carbohydrate. It can be dissociated into two subunits of 50,000 kDa (Luke and Coffey, 1994). As seen in the results of the present study, *Uraeotyphlus* male Mullerian gland does possess ACP, and its activity is 10 times that in the serum. But, as the indication of its presence is based on native gel electrophoresis, and using the specific substrate, its MW could not be inferred and, therefore, a comparable protein in the SDS-PAGE could not be identified. Thus, there is scope for pursuing research on ACP of caecilian male Mullerian gland so as to find the evolutionary relationship between caecilian male Mullerian gland and mammalian prostate in respect of this enzyme.

One of the interesting observations of the present study is the presence of antigenically similar proteins in the Mullerian gland, liver and or testis. This can lead to several speculations, like i) the protein is basically synthesized in liver and carried in blood for sequestration into Mullerian gland and testis, ii) proteins are synthesized in the liver and carried in blood for sequestration into Mullerian gland, iii) one or more protein like, for example, MW 24 kDa may be synthesized in the Mullerian gland, carried in blood and sequestered into the testis. Thus, the present study shows, for the first time, that Mullerian gland is the source of several proteins including glycoproteins, acid phosphatase and Ca$^{2+}$- and Na$^{+}$-K$^{+}$ ATPases, and some of these proteins originate in the liver, and some others are made available to the testis. ATPases are the molecular machine that uses the energy of hydrolysis of ATP for the coupled active transport of Na$^{+}$, K$^{+}$ and Ca$^{2+}$ across the plasma membrane. They are essentially energy transducing ion pumps. They also act as signal transducers from plasma membrane to the nucleus (Xie and Askari, 2002). Their presence in the caecilian male Mullerian gland indicates the gland to be metabolically very active and there occurs considerable signal transduction.
The study also confirms the secretion of a monosaccharide by the Mullerian gland. Wake (1981) had earlier shown in *Dermophis mexicanus* and *Typhlonectes compressicauda* either glucose or fructose to be present in the Mullerian gland, the two occurring during different reproductive status of the animal. But the study was based on spot tests. In the present study, the analysis is based on paper chromatography. In most of the mammals, the seminal vesicles are responsible for secreting fructose as the seminal sugar and adding it to the semen. In some mammalian species the sugar is glucose. This sugar forms one of the major extracellular energy substrates to facilitate sperm motility in the female tract (Mann and Mann, 1981).

Thus, the present study proposes similarity between the roles of caecilian male Mullerian gland and mammalian male accessory reproductive gland, and ascertains the Mullerian gland to be a male accessory reproductive gland.

**REFERENCES**


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Table 1. ACP activity in Mullerian gland and serum.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tissue</th>
<th>ACP activity (μM inorganic phosphate formed mg⁻¹ protein h⁻¹)</th>
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<tr>
<td>1</td>
<td>Mullerian gland</td>
<td>0.1053 ± 0.0031</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>0.0167 ± 0.0008</td>
</tr>
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p< 0.001
FIGURE LEGENDS

Fig. 1. Electrophoretic resolution of Mullerian gland proteins of *Uraeotyphlus narayani* in Coomassie brilliant blue-stained SDS-PAGE gel. Lane M, standard molecular weight marker proteins; lane MG, Mullerian gland proteins.

Fig. 2. Coomassie brilliant blue-stained SDS-PAGE gel showing proteins of Mullerian gland (lane MG), testis (lane UT), liver (lane UL) and serum (lane US) of *U. narayani*. Molecular weight markers are shown on the left (M).

Fig. 3. PAS-stained SDS-PAGE gel showing proteins of Mullerian gland (lane MG), testis (lane UT) and liver (lane UL) of *U. narayani*.

Fig. 4. Electrophoretic resolution of Mullerian gland proteins of *U. narayani* in Coomassie blue-stained SDS-PAGE gel during the two phases of testicular activity. Lane M, standard molecular weight marker proteins; lane MGl, Mullerian gland during the period of active spermatogenesis; lane MG2, Mullerian gland during testicular quiescence.

Fig. 5. Densitometric scan data on the various protein fractions in the SDS-PAGE gels.

Fig. 6 Double immunodiffusion pattern showing the reaction of anti-Mullerian gland serum against proteins of testis, liver, Mullerian gland and serum of *U. narayani*. A, anti-Mullerian gland serum; MG, Mullerian gland; UL, liver; US, serum and UT, testis.

Fig. 7a. Immunoelectropherogram showing cross-reactivity of anti-Mullerian gland serum against the protein extracts of Mullerian gland (MG) and testis (UT) of *U. narayani*.

Fig. 7b Immunoelectropherogram showing cross-reactivity of anti-Mullerian gland serum against the protein extracts of Mullerian gland (MG) and liver (UL) of *U. narayani*.

Fig. 7c Immunoelectropherogram showing cross-reactivity of anti-Mullerian gland serum against the protein extract of serum (US) of *U. narayani*. 87
Fig. 8 Immunoreactivity, detected on immunoblot analysis, to anti-Mullerian gland serum of proteins of Mullerian gland (MG), testis (UT), liver (UL) and serum (US) of *U. narayani*.

Fig. 9 Localization of acid phosphatase in the Mullerian gland extract of *U. narayani* in native PAGE gel.

Fig. 10 Paper chromatogram of monosaccharide (M) of the Mullerian gland extract, co-run with standard glucose (G) and fructose (F).