SEPARATION OF THE PROTEINS IN THE EPIDIDYMAL FLUID OF THE GOAT

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ABSTRACT

It is believed that epididymal secretions provide the microenvironment necessary for the maturation of spermatozoa. Many proteins have been identified as important constituents of epididymal fluid and most of these are considered to be secreted by the blood serum. Information on epididymal fluid and its protein constituents is limited in the goat and this study is an attempt to characterise the proteins present in the epididymal fluid of indigenous goats.

The cauda epididymis was incised and fluid was collected into micro-capillary tubes. Capillary tubes were centrifuged in a micro-haematocrit centrifuge and the supernatant was collected. The supernatant was re-centrifuged at 14,000 rpm 4°C to obtain spermatozoa-free fluid. Goat blood serum was collected for comparison. The proteins of the epididymal fluid and serum were fractionated by SDS-PAGE. The gels were fixed and stained with Coomassie blue dye and destained.

Eighteen distinct protein bands were detected in epididymal fluid and nine were detected in serum. Five bands in epididymal fluid showed mobility patterns similar to that of serum. The most prominent band observed in both epididymal fluid and serum was the albumin band.

Further studies using such techniques as immuno-electrophoresis are necessary to confirm the relationship between the serum and epididymal proteins.

INTRODUCTION

The primary reproductive organ in the male is the testis, which consists of a series of coiled seminiferous tubules responsible for producing spermatozoa, the male gametes. Although the spermatozoa are produced in the testis, their final maturation occurs during transit through the epididymis which is an organ located adjacent to the testis. It is well established that spermatozoa recovered from the testis are immotile and are incapable of fertilising an ovum (Mann, 1964). During transit through the epididymis, which takes approximately 2 weeks, spermatozoa acquire the capacity for fertility and motility. The exact mechanisms involved in epididymal function have not been fully elucidated, but it is generally accepted that epididymal fluids provide the microenvironment needed to acquire motility and fertilising ability. Motility is a unique feature of spermatozoa and they are capable of exhibiting a wide range of movements including vibration, rotation and forward progression. Of these the most important type of movement is forward progression and in certain species a protein designated as forward Motility-Stimulating Factor has been identified (Mandal, Banerjee, and Majumder, 1989). Although spermatozoa acquire the potential for

movement, it is also well established that spermatozoa remain quiescent as long as they are within the epididymis. This feature has also been associated with certain epididymal proteins. Notable among these are "immobilin" identified in the rodents and "quiescence factor" reported in the cattle. Other proteins such as 135 kD manosidase, procathapsin L , and "anti-sticking factor", have been identified as important constituents of the epididymal fluid.

The composition of the proteins of the epididymal fluid have been studied in different species and it has been found that they are mainly derived from blood serum. (Quinlivan, 1968; Alumot, Lensky and Schindler, 1971, Dacheux and Paquignon, 1989). However, there is a dearth of information on epididymal proteins in the goat. The purpose of this study was to separate the epididymal proteins in goat, and to compare them with those of goat blood serum.

The method used was sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) which is one of the most widely used method for separating protein mixtures and for determining their molecular weight. (Wilson and Goulding, 1991).

MATERIALS AND METHODS

Preparation of epididymal fluid and serum:

Testes with the epididymides were collected from mature goats and brought to the laboratory at 4°C . The epididymis was dissected form the testis . The tail of the epididymis was incised and epididymal fluid was collected into the micro-capillary tubes and centrifuged using a micro-haematocrit centrifuge (Hawksley-England) for ten minutes. The supernatant was collected into micro-centrifuge tubes and re-centrifuged at 14,000 rpm / 4°C for ten minutes to obtain spermatozoa free epididymal fluid which was stored at -20°C . Goat serum samples were also collected and prepared as described by Herbert(1978) for the purpose of comparison.

Preparation of samples for SDS- PAGE:

Epididymal fluid and serum were first diluted with 0.5M Tris-HCl (pH 6.8) and then further diluted with sample buffer (0.5M Tris-HCl 6.8,10% SDS and ditheriothriol) to obtain the final dilutions. The final dilutions of the epididymal fluid were 1:16, 1:32, and 1:64 while the dilutions of the serum were 1:40, 1:80 and 1:160. Samples were heated for three minutes in boiling water before application.

Molecular weight markers ranging from 12,000 kD to 78,000 kD were prepared according to the manufactures recommendations (BDH Laboratory Supplies, England) and used together with other samples.

Electrophoretic separation of proteins:

Samples were subject to electrophoresis by SDS-PAGE (Mini-PROTEAN II Cell , BIO-RAD Chemical division, California) with different gel concentrations as 12%T, 10%T and 7.5%T under different electrophoretic conditions. Gels were stained for 1/2 hr with 1% Coomassie blue R-250 in fixative which was a mixture of 40% methanol and 10% acetic acid and destained with 40% methanol / 10% acetic acid for 4 to 6 hours to remove background stain.

RESULTS AND DISCUSSION

The 10%T gels gave a good separation of epididymal and serum proteins. Although the recommended power conditions for optimal resolution are 100V and 200V for the stacking and separating gel respectively, these conditions did not give a good resolution of the protein bands. As suggested by several workers, the voltage was reduced sequentially and it was found that 80V for 15 min and 160V for 45 min for the stacking and separation gels respectively were the best electrophoretic conditions, which gave optimum resolution in the present study. The epididymal fluid at 1:32 dilution and serum at a dilution of 1:160 gave good resolution of protein bands.

In the epididymal fluid 18 distinct protein bands were identified. Alumot Lensky and Schindler, (1971) found 17 protein bands in the epididymal fluid of the ram while Koskimies and Kormano, (1975) found 16 protein bands in the rat. These differences may be due to species variation or due to the usage of different techniques such as disc electrophoresis (Alumot, Lensky and Schindler, 1971) and step-gradient gel electrophoresis (Koskimies and Kormano, 1975). The previous studies did not use molecular markers and therefore did not relate the protein bands of the epididymal fluid to molecular weights. Due to this, it was not possible to give an identity to the additional protein band present in the goat epididymal fluid. Nine distinct protein bands were identified in goat serum. Five protein bands of the epididymal fluid showed a similar mobility to those of serum indicating that they were most likely to be derived from the serum. Contrary to these findings, Alumot Lensky and Schindler, (1971) found 14 precipitation bands in blood serum of ram with antiserum prepared against the ram epididymal fluid. In studies using the boar, it was reported that only a few of the proteins in epididymal fluid showed mobility patterns comparable to those of boar serum (Dacheux and Paquignon, 1989). Of all protein bands in the epididymal fluid, the most prominent band was in level with the albumin band in the serum samples. It was also in line with the 66 kD molecular weight marker. These observations suggest that the major protein in the epididymal fluid of the goat is albumin. In other species too, albumin has been identified as the most prominent band of the epididymal fluid (Dacheux and Paquignon, 1989; Sedlakova , Dostal and Matousek, 1968; Alumot,Leusky and Schindler, 1971).

The function of albumen in epididymal fluid is uncertain. Even though the exact structure of the forward motility-stimulating factor has not been identified, Mandal, Banerjee and Majumder, (1989) showed that it was a heat stable glycoprotein present in both epididymal fluid and the blood serum which was not one of the major

serum proteins such as albumin. From the present results it can be seen that there are three protein bands above the 78,000 kD molecular weight marker and it is possible that one of these is 135 kD manosidase.

CONCLUSIONS

Some of the epididymal proteins showed similar mobility to proteins in the serum suggesting that they are derived from serum . Further studies using such techniques as immuno-electrophoresis are necessary for confirming the identity of these proteins.

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