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Review

Mammalian epididymal proteome

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ABSTRACT

In all mammalian species, the final differentiation of the male germ cell occurs in the epididymal duct where the spermatozoa develop the ability to be motile and fertilize an ovum. Understanding of these biological processes is the key to understanding and controlling male fertility. Comparative studies between several mammals could be an informative approach to finding common sperm modifications which are not species-specific. The new global biological approaches such as transcriptomes and proteomes provide considerable information which can be used for such comparative approaches. This report summarizes our proteomic studies of the epididymis of several mammals, including humans.

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1. Introduction

Epididymal function is essential for the fertility of male mammals because their sperm is infertile when they leave the testes and only acquire the ability to fertilize an ovum during passage through the epididymides. It is also essential that the epididymides accumulate and store sperm as, depending upon species, it takes 0.5–2 days for the testes to produce the number of sperm in a normal ejaculate, and in a competitive mating system males may inseminate up to 50 females in a day (Jones, 1999; Jones et al., 2007). The efficacy of sperm storage in the epididymis is so high that fertile sperm can survive in an isolated epididymis for several days at 4 °C (Guérin et al., 2003).

The epididymis is a very long duct which receives testicular sperm via the efferent ducts. In eutherian and marsupial mammals the duct is differentiated into about six structurally distinct segments, indicating a well-developed division of labor. Although all mammals have an initial segment of the epididymis with distinctive characteristics, there is variation between species in the structure and extent of the different segments, suggesting some variation in post-testicular sperm maturation and storage (Jones, 2002). The division of labor through the epididymis has been confirmed in studies of epididymal physiology and sperm modifications along the duct, and these findings have lead to the paradigm that the maturational changes in sperm in the epididymis are the result of sequential changes in their milieu, particularly the proteins secreted by the epididymal epithelium (Dacheux et al., 2003). In view of the variations in epididymides between species mentioned above, it is considered that there must be conserved aspects and variations between species in the changes in protein composition throughout the epididymis, presumably reflecting the occurrence and relative significance of sperm maturation and storage between species. This short review therefore compares our findings regarding the proteome and secretome of epididymal fluid in mammalian species: i.e. the stallion, ram, boar and human.

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2. Characteristics of intra luminal epididymal proteomes

Most of the studies analyzing the proteins of epididymal fluids have been performed on samples obtained either from tubule micropuncture (Turner et al., 1995, 1999) or from microperfusion techniques (Druart et al., 1994; Syntin et al., 1996; Fouchecourt et al., 2000; Dacheux et al., 2006). The epididymal fluid provides a milieu for the gamete analogous to blood plasma providing a milieu for cell tissues. Due to the presence of the blood-testis and blood-epididymis barriers, most of the blood proteins are not found in epididymal plasma (albumin, transferrin and certain others being exceptions). In contrast to the stable concentration of blood plasma proteins, the protein concentration in epididymal fluid varies greatly along the duct: from 2 to 4 mg/ml in the initial segment of the epididymis, a maximum of 50 to 60 mg/ml in the distal caput and 20 to 30 mg/ml in the more distal regions of the organ (Fig. 1C). For most of the species studied, these variations in protein concentration follow the changes in water content of the fluid as assessed by changes in sperm concentration (Fig. 1A).

Several hundred epididymal proteins have been described electrophoretically and some have been identified. There is a wide range of dynamics in the abundance of these proteins (probably around 10 orders of magnitude). About 15–20 proteins make up more than 60–80% of the total protein concentration. The most common proteins found are lactoferrin, procathepsin D, NCP2 (HE1, CTP, cholesterol transfer protein), GPX (glutathione peroxidase), beta-N-acetyl-hexosaminidase, mannosidase, galactosidase, PGDS (prostaglandin D2 synthase), clusterin, CRISP (cystein-rich secretory protein) and E-RAPB (epididymal retinoic acid-binding protein).

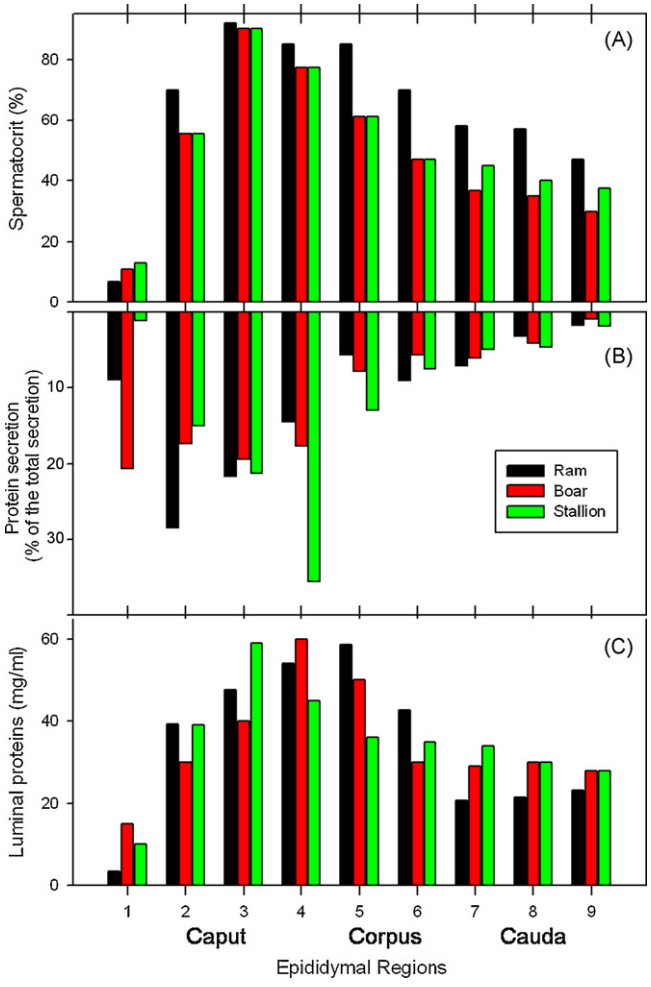

Fig. 1. Spermatocrit (A), protein secretion (B) and concentrations of luminal proteins (C) in the epididymal fluid from nine regions of the epididymis (1–4: caput; 5–6: corpus; 7–9: cauda) for three species (ram, boar and stallion) from (Syntin et al., 1996; Fouchecourt et al., 2000) and unpublished data.

The protein composition changes continuously throughout the duct, independently of the protein concentration in the fluid. The concentrations of the major common proteins cited above vary between species (Fig. 2). Lactoferrin, mannosidase, PGDS and albumin are present in high concentrations in the stallion, boar, ram and human, respectively, but GPX and PGDS are virtually absent in humans and boar, respectively.

Most of the epididymal proteins are characterized by their numerous isoforms which result from their high degree of glycosylation. The p*I* of these multi-isoforms can vary widely, ranging from pH 3 to 8 for the same protein (i.e. RNase 10 in the boar, Fig. 3). The degree of glycosylation for the same protein can be different according to the epididymal region, e.g. clusterin and PGDS in the horse, where the number of isoforms is different between the anterior and the posterior part of the organ (Fig. 3), or RNase 10 (Train A) which is different between species for the boar and the ram (Fig. 3).

3. Dynamics of epididymal fluid proteomes

The spatial changes in the composition of luminal proteins are the result of two opposite activities of the epithelium: protein secretion and protein absorption throughout the epididymal duct. In the anterior part of the epididymis, the epididymal fluid is composed of a mixture of testicular and epididymal proteins. Most of the proteins originating from the testis, such as albumin, transferrin, testicular clusterin and PGDS (Fouchecourt et al., 2000) are reabsorbed in the efferent ducts (Clulow et al., 1994). The rapidity of their absorption is species-specific and generally almost all are absent in the posterior part of the epididymis, except in humans in which albumin and transferrin are still present in large quantities.

The epididymal epithelium has high protein synthesis and secretion activity, activity being high both in the rates of protein synthesis and secretion and in the variety of proteins secreted. The anterior part of the epididymis is the most active (Figs. 1B and 2). As for the protein concentration, from 70% to 80% of the secretome is composed of 10–20 of the major secreted proteins present in the luminal fluid (Fig. 4). Most of the luminal proteins are secreted by the epithelium, but some, such as ACE, are released from the sperm surface by an unknown sheddase in an anterior part of the epididymis (Fig. 6) (Gatti et al., 1999; Metayer et al., 2002; Thimon et al., 2005).

Among the different proteins secreted, the same protein can be secreted in the same region of the epididymis in different species, for example PGDS, GPX and clusterin in the anterior part, and glucosidases in the middle part. Clusterin is secreted at a greater rate than the other proteins and can represent 30–40% of the all the proteins secreted. This clusterin can be sequentially secreted under different isoforms in different parts of the epididymis as in the stallion and the ram (Figs. 2 and 3). Some highly secreted proteins are characteristic of a species, for example, lactoferrin in the stallion, PGDS and GPXin the ram and RNase 10, mannosidase and hexosaminidase in the boar (Fig. 2).

In humans, in contrast to other species, few changes in pattern of protein secretion occur throughout the epididymis, a finding which correlates with the low degree of structural differentiation of the epididymal epithelium along the duct (Holstein, 1969).

Variations in luminal protein concentrations, controlled by secretory and absorption activities, are modulated for each species by the length of the epididymal duct involved in the two activities, i.e. the flow rate of the luminal fluid, enzyme degradation or the binding of protein on the sperm membrane. All these parameters