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Epididymal protein markers and fertility

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ABSTRACT

The last stages of male gamete differentiation occur outside the gonad in a specific environment controlled by the epididymal epithelium. All the fundamental characteristics of a fertile spermatozoon are acquired sequentially during transit through the epididymal tubule. Full understanding of the mechanisms involved in these gamete modifications is a key to understanding and controlling such important stages in male fertility. With the development of new large scale technologies, large amounts of information give hope of identifying the fundamental elements involved in such cellular events and of being able to obtain some markers predictive of male fertility that would be valuable both in human and/or animal reproduction.

1. Introduction

The fertility of the male gamete is achieved only after full transit through the male genital tract and can be characterized by 1) the ability of spermatozoa to migrate through the female tract, 2) binding to and penetration through the zona pellucida, 3) fusion with the oocyte membrane and 4) the ability to result in a viable embryo.

For all mammals, this fertilization potential of the spermatozoon is the result of discrete post-gonadal differentiation which occurs during transit through the several meters of the epididymal tubule. Furthermore, these modifications occur in a male gamete which loses its transcriptional and translation abilities during the last stages of spermatid differentiation.

During "epididymal maturation" the spermatozoa are contained in a specific environment which has an essential role in controlling or inducing the final maturation of the spermatozoa. Many investigations have been undertaken over more than sixty years in order to understand the epididymal mechanisms involved in the transformation of an immature to a mature fertile spermatozoon. In the last ten years, studies using new techniques such as genomic, transcriptomic and proteomic approaches have provided new results and new perspectives in the understanding of the events occurring on/in the sperm and their epididymal environment.

Furthermore, as epididymal sperm maturation occurs in all mammals, comparative studies will be able to identify common fundamental mechanisms of post-testicular differentiation between species.

Is it therefore now possible to obtain predictors of male fertility from the results of these many investigations into the epididymal activity and sperm composition?

2. The epididymal tubule: a storage organ for viable spermatozoa

The first function of the epididymis, which is directly involved in male reproduction, is the storage of spermatozoa produced by the testis. Depending upon the species, the testes of mammals produce only sufficient spermatozoa for 0.5 ± 2 ejaculates per day (Jones 1999). According to

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the mode of sexual behavior and mating rate, there is considerable variation between species in the proportion of extra gonadal sperm stored in the epididymis, (Jones et al., 2007). For example, the spermatozoa stored in the cauda epididymidis of the bull, stallion, ram and boar represent, 9, 14 and 6 days of the testicular sperm production, respectively (Amann et al., 1976). The storage is the result of both the volume capacity of the total luminal space of the tubule and also associated with the high level of sperm concentration which occurs as soon as the testicular fluid enters the efferent duct and the anterior part of the epididymis. Active resorption of the water from the luminal fluid by the epididymal epithelium lining the tubule increases the sperm concentration from 10^6 to 10^9 sperm atozoa/ml from the rete testis to the caudal epididymis.

The second important function of the epididymis in relation to male fertility is its capacity to preserve the viability of these stored gametes for one or two weeks. Such preservation is principally linked to inhibition of sperm motility associated with a reduction in their metabolic rate (Jones and Murdoch 1996). Several external influences are involved in this control of sperm motility. The first is reduction in the scrotal temperature, which is lower than that of the core body and testis (Brooks 1973). Preservation of such a low temperature is so important that an increase in the scrotal temperature by only 2 °C for 4 days induces a decrease in sperm motility and an increase in the rate of embryonic mortality after artificial insemination (Mieusset et al., 1991). The second factor which represses sperm motility is linked to the low oxygen content in the epididymal fluid and tissue (Free et al., 1976), the absence of glucose in the luminal fluid (Scott et al., 1963) and variation in the ionic composition of the epididymal fluid such as bicarbonate and Ca^{++} concentrations involved in control of the motility of gametes (see review by Dacheux and Dacheux 2014). The protective effect of the epididymal environment on stored sperm is so efficient that fertile spermatozoa have been retrieved from epididymides stored at 4 °C for several days after the death of an animal (Fernandez Abella et al., 2015).

The third important activity of the epididymis is to control the final post- gonadal stages of sperm differentiation which transform the immotile, infertile testicular spermatozoon into a motile, fertile male gamete. The interactions are so complex that no attempt at in vitro maturation of testicular spermatozoa has been successful to date. Understanding the composition of the content of the epididymal lumen is expected to be a key to understanding the mechanisms involved in sperm survival and maturation in the epididymis.

3. The epididymal tubule with active epithelium which continuously modifies the medium surrounding sperm

During transit through the epididymal tubule, spermatozoa are surrounded by a specific and continuously modified medium. This specificity of the epididymal fluid composition results from the presence of a robust blood barrier which prevents diffusion and exchange with the serum proteins. A break in the integrity of this barrier, such as during inflammation (Gregory and Cyr 2014), very rapidly causes the infertility in animals resulting from an immune response.

The complexity of the composition of luminal fluid is the result of two antagonistic cellular mechanisms originating from epithelial cells, i.e. active secretion and reabsorption throughout the epididymal tubule. As almost all the components from the rete testis fluid are reabsorbed as soon they pass from the first part of the epididymis, most of the components present in the epididymal fluid are the result of active secretion by the epithelial cells throughout the epididymal tubule.

Identification of proteins secreted by the epididymis has been the aim of several hundred studies since the 70–80' s and the first epididymal protein cloned by Brooks (Brooks et al., 1986). However, with the use of large scale identification technologies, such as proteomics, transcriptomics and genomics during the last decade, several thousand proteins have been characterized in the epididymis and several hundred identified as being secreted.

3.1. Epididymal tissue activities are highly regionalized and species-specific

In mammals, the epididymis is composed of three regions, the anterior or caput, the median or corpus and the posterior or cauda, but can be also subdivided into several segments as in rodents (Jelinsky et al., 2007). However, wide differences in size and anatomical segmentation exist between species. High-throughput microarray studies of the mRNA of epididymal tissues from several mammalian species (e.g. the mouse, Johnston et al., 2007; rat, Jelinsky et al., 2007; human, Zhang et al., 2006; Thimon et al., 2007; Li et al., 2008 and boar, Guyonnet et al., 2009) have confirmed that this organ is functionally highly regionalized and the regionalization is more complex than the anatomical regions (Fig. 1).

In spite of the difficulty of comparing these transcriptomic studies due to the different technologies used, several hundred genes have been shown to be common in the four species studied (Guyonnet et al., 2009). Among the several thousand genes mapped in these studies, about 20–30% of them have been shown to be highly regulated through the epididymis and most of them are species-specific (Fig. 2). Of these, 3–5% are epididymis-specific, with very restrictive segment expression, such as the beta defensin gene family (Fig. 3) (Jelinsky et al., 2007) (Fig. 3), and RNAse 10 (Penttinen et al., 2003; Castella et al., 2004a).

In addition to these global studies of mRNA throughout the epididymis, recent analyses have been performed for non-coding RNA such as the micro RNA involved in gene-regulating expression. Of the hundreds of miRNA identified, 10–25% display expression levels which differ significantly between epididymal regions and only about 21% of them have been found to be conserved across the different species studied (Fig. 4) (human, Belleannee et al., 2012; rat, Ma et al., 2012; mouse, Nixon et al., 2015a). Most of these conserved miRNA were found to be expressed in all epididymal regions and in all species, suggesting that they

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