The influence of high osmolality upon the maturation of spermatozoa in the boar epididymis

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Introduction

Mammalian spermatozoa mature as they pass through the epididymis of the male reproductive tract. The interaction between spermatozoa and epididymal fluid components is an important part of this maturation process and the epididymal epithelium generates and maintains this fluid.

The high osmolality environment in the epididymis is thought to be instrumental in the acquisition of solutes by spermatozoa. By regulating their volume post ejaculation, these solutes enable spermatozoa to enter the comparatively hypo-osmolar environment of the female reproductive tract without developing morphological abnormalities.

Some mammalian species, including the rat, mouse, hamster, cow and sheep are known to have epididymal fluid with an osmolality significantly higher than that of mammalian blood. Little is known of the osmolality of boar epididymal fluid and this investigation aimed to find a value for this and compare that to known, published values for the osmolality of pig blood (Pastor-Soler *et al*., 2001).

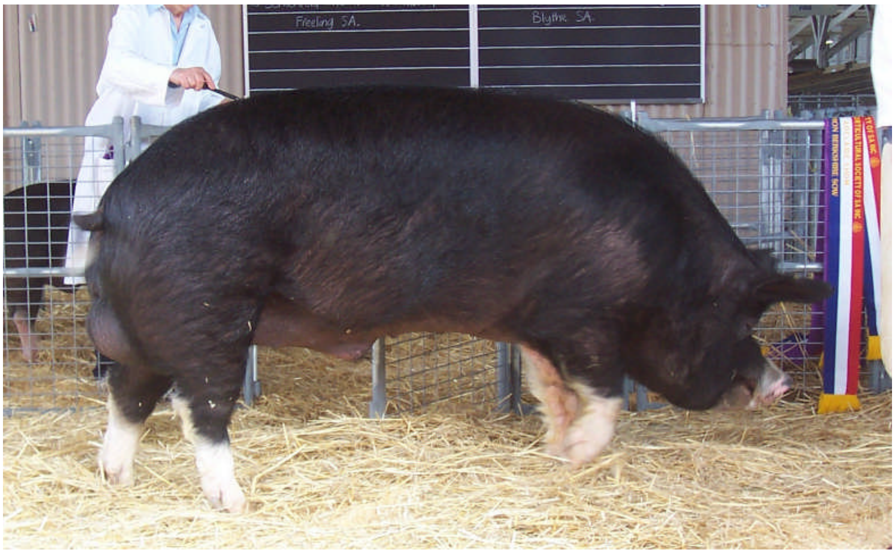

Figure 1. Champion Berkshire boar, Royal Adelaide Show 2005, Scott Davis, Wikimedia Commons.

The Epididymis

The epididymis has three distinct regions and the cells of the epididymal epithelium vary from region to region, creating a changing fluid microenvironment which enables step by step spermatozoa maturation. The three layers are defined as the caput (head), corpus (body) and cauda (tail), shown in figure 1 and based on differences found in the gross morphology and epithelium structure (Dacheux *et al*., 1998).

The fluid microenvironment creates the ideal solution for spermatozoa maturation via secretory cells such as principal cells, endocytic cells such as apical cells and the communication of these (and other) epithelial cell types with spermatozoa.

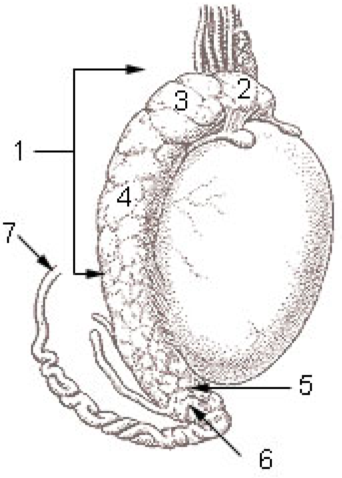

Figure 2. The epididymis; 2-3: Caput, 4: Corpus, 5-6: Cauda epididymis. Wikimedia Commons

One of the most notable functions of the epididymal epithelium is the generation of a substantial increase in the concentration of solutes within the epididymal fluid due to water reabsorption (90% of the water originally in boar epididymal fluid is reabsorbed). This is possible due to the passage of water through ion channels and aquaporins.

Ion Channels

One theory on water removal from the epididymal lumen states that ions are actively pumped out of the epididymal lumen, setting up an osmotic gradient. An active sodium pump moves sodium ions across the epididymal epithelium, out of the lumen. The sodium ions passively diffuse across the apical surface of the epididymal epithelium and pass across the basolateral membrane by active transport. It has been suggested that this active transport occurs by a sodium-potassium pump (shown in figure 2).

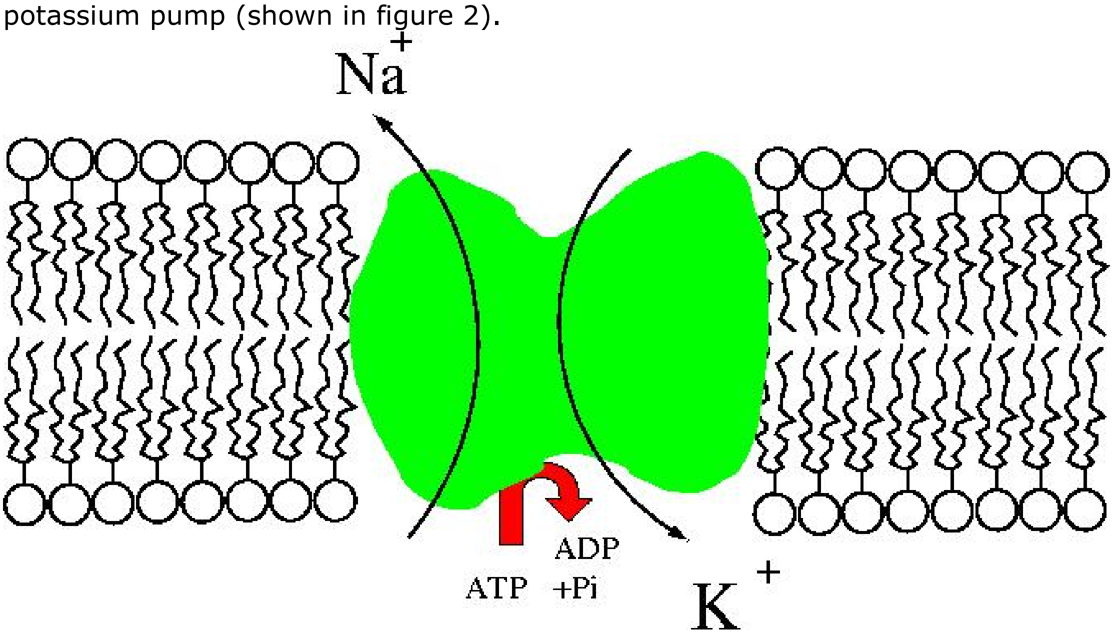

Figure 3. A sodium-potassium pump working across a lipid bi-layer membrane. GNU Free Documentation License.

It has been shown that the sodium-potassium pump is more active in the *ductuli efferentes* and less active in the distal regions of the epididymis, suggesting that water reabsorption becomes less important further along the tract. Water is thought to travel predominantly through inter-cellular spaces, known as the "paracellular route" and only to a lesser extent through the epithelial cells, the "transcellular route".
More recent studies tend towards a multiple ion-pump mechanism for water removal based upon the above theory that water will travel out of the epididymal lumen, up an osmotic gradient. Three mechanisms have been found which facilitate the secretion of ions across the epididymal epithelium. These are a Na^+H^+ exchange system, a Cl^-HCO^- exchange system and a $Na^+K^+2Cl^-$ symporter.

A comparison between the epididymal epithelium and the renal tubule epithelium in the kidney seems a logical step, considering the water-controlling properties they both display. Levine and Marsh (1971) state that when raffinose (a sugar naturally present in the diet from vegetables and grains) is present in the renal tubule the renal epithelium becomes impermeable to water and they hypothesise that organic acids and bases play this role in the epididymal lumen. When these are present in high concentrations, the epididymal epithelium becomes impermeable to water but ions are still able to pass across both by diffusion and active transport. It is then that an osmotic gradient is able to build up, resulting in water travelling out of the lumen, up this osmotic gradient as soon as the epithelium regains water permeability (Turner, 1991).

Aquaporins

Lipid bilayer membranes allow water to pass through them by simple diffusion however this is a slow process. Proteins called aquaporins allow 10 to 100 times more water to pass across the membrane.

Aquaporins are water transport proteins, found in membranes involved in many different mammalian systems including the nervous, respiratory and reproductive systems. All aquaporins contain related amino acid sequences. The specific mechanisms by which each of the aquaporins operate are yet to be found, however it is acknowledged that some allow the passage of water and prevent the passage of ions and small uncharged molecules (AQP1 is an example of such an aquaporin) whilst others are thought to operate as ion or small molecule channels, but this has not been proven.

The specificity which allows some aquaporins to transport water and no other small molecules or ions is essential to their operation in membranes which form a barrier between liquids of different osmolalities. The process of water transport by aquaporins has a low activation energy which indicates that the water molecule forms a single-file column as it passes through the bilayer. There are theories based on this which suggest that the positioning of charged areas within the aquaporin interact favourably with water molecules, but block the passage of ions (Agre, 2004).

AQP1, AQP2, AQP7, AQP8, AQP9 and AQP10 have been found in the epididymis. They are known to facilitate passive diffusion of water, but it has been hypothesised that they may also be involved in the active transport process although this has not been proven and is widely disputed.

The location and regulation of each of the aquaporins indicates the secondary nature of this mechanism for water reabsorption, compared to that of ion movement. There are no aquaporins present in dog seminiferous tubules or rat testes which indicates the function of these proteins being specifically to assist the reabsorption of water from the efferent duct and epididymis. AQP1 is exclusively a water channel and is not thought to be regulated but AQP9 (capable of facilitating the diffusion of water and small uncharged solutes) is strongly regulated by oestrogens and androgens. This indicates that water regulation is secondary to electrolyte regulation in the epididymis (Agre *et al*, 1995).

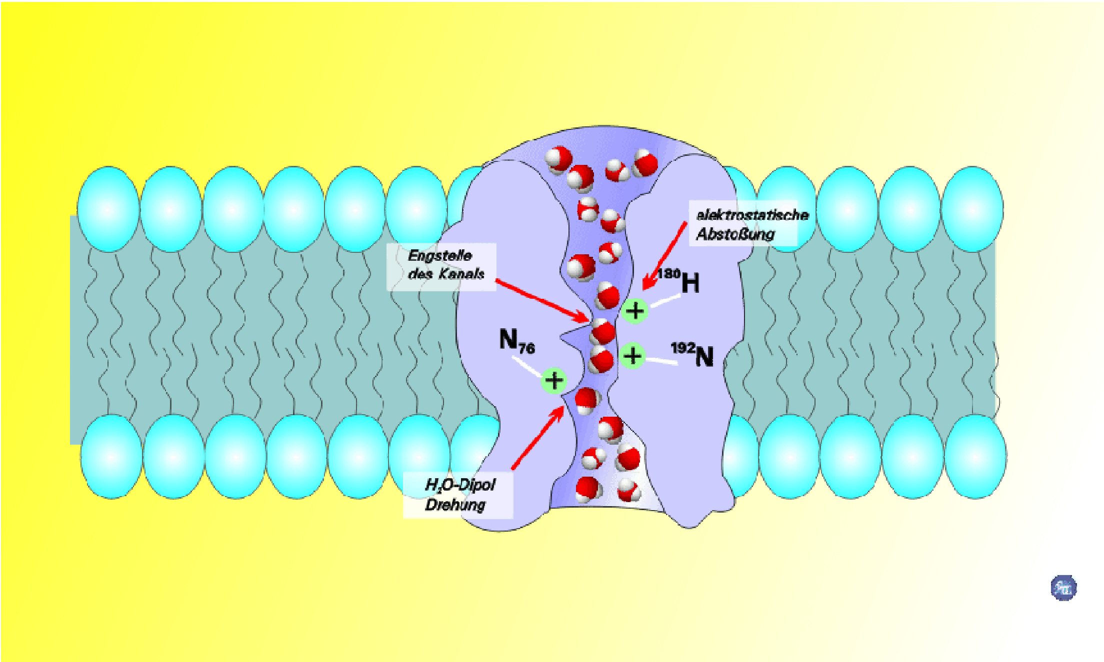

Figure 4. Schematic depiction of water movement through the narrow selectivity filter of the aquaporin channel. Wikimedia Commons

Collecting and Analysing Boar Epididymal Fluid

Boar epididymal fluid samples were aspirated *post mortem* from the *cauda epididymides* of five boars. Each epididymis was presented with the whole testis attached. The epididymis and deferent tubule was detached from the testis and tissue was cut away until one single loop of the cauda epididymis was exposed. This was cut so an isolated region of epididymis which was entirely intact, but with two open ends was created. A blunted hypodermic needle was inserted into the corpus end of the isolated region of epididymis and air was pumped into the lumen. This increased the pressure in the lumen so that fluid was ejected from the open cauda end of the isolated region. Fluid collection was unsuccessfully attempted from the corpus and caput regions of each epididymis.

A ‘5520 VAPRO’ vapour pressure osmometer was used to find the osmolality of the epididymal fluid samples. This deduces osmolality by a ‘depression of dew point’ mechanism. The 10µl sample is heated in a sealed chamber containing a thermocouple, until completely vapourised. As the sample cools, the vapour will return to liquid form. The point at which this occurs is referred to as the ‘dew point’ and its temperature is detected by a thermocouple. The difference between the ambient temperature and the dew point temperature is the ‘dew point temperature depression’ and is used by the vapour pressure osmometer to calculate the osmolality of the sample.

The mean osmolality was found to be 348.53mOsm, with a standard deviation of 8.25 and a range of 330mOsm to 365mOsm. The normal osmolality for pig blood is known to be 290 (+/- 3) mOsm. The osmolality of boar epididymal fluid is therefore significantly higher than that of pig blood.

Discussion

Current theories on the acquisition of high osmolality vary considerably and whilst it is not possible for every detail in each of them to be correct simultaneously, the main concepts do not completely contradict one another. It has been shown that 70% to 90% of the water initially in the epididymal fluid is reabsorbed. Mechanisms suggested for this include active ion transport creating an osmotic gradient and the involvement of aquaporins, as described previously. The control of aquaporins is pivotal to this theory. Aquaporins are gates through which water can travel easily, when an osmotic gradient exists. If those gates were permanently open, no osmotic gradient would be able to be established. However, aquaporins are able to make a lipid bilayer membrane highly permeable (open gates) or predominantly impermeable (closed gates) to water.

The hypothesis here involves a process of water removal in two phases. In the first, aquaporins are impermeable to water and there is a high level of active ion transport across the epididymal membrane. A high concentration of ions is built up outside the epididymal lumen and an osmotic gradient is therefore established. The second phase coincides with the high water permeability of aquaporins. Water is able to move freely across the epididymal membrane and passes up the osmotic gradient, out of the epididymal lumen. It has not been established whether these 'phases' occur in time, or in distance as the spermatozoa pass along the epididymis. The trigger which changes the epididymal epithelium between these two phases is likely to be one of the compounds already described as having an effect on water transport or epithelium control. These include androgens, aldosterone, prostaglandins, diuretics and oestrogens.

The removal of water, whilst contributory, cannot be the complete reason for the high osmolality of epididymal fluid. The flow of fluid immediately outside the epididymis would have to be rapid in order for the water to be taken away or aquaporins would regain permeability when there is a large amount of water immediately outside the epididymis. No osmotic gradient would exist. For this velocity of flow, the fluid would need to be in close contact with a blood supply and this is not the case. When the epididymides were dissected in order to obtain fluid for this experiment, it was noted that there was very little vasculature immediately adjacent to the epididymis.

The contents of the epididymal lumen include lipids, sugars, proteins, alcohols and spermatozoa in a dense milieu. Alone in a water-based solution the individual particles may not be expected to create a hyper-osmotic environment in relation to water, however in the very high concentrations found in the epididymal lumen it is predicted that their osmotic effect would be significant. This combined with the water removal described above could be sufficient to create the high osmolalities found in the epididymis.

It is important to be aware that the mechanisms theorised here are applicable to the cauda epididymis only because it is known that the fluid microenvironment of the epididymis changes from region to region and it is subsequently thought that the mechanisms controlling the lumen environment must also change from region to region. This investigation was only able to obtain osmolality values from the cauda epididymal fluid and as a consequence has only been able to suggest mechanisms for this region.

It is also important to note that whilst high osmolality of epididymal fluid in relation to blood has been recorded in a number of mammalian species, specific mechanisms, molecules and interactions vary between species.

Further Reading

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Author Profile

Carrie is 22 years old and graduated from the School of Biosciences with a 2:1 BSc (hons) in Animal Science in 2008, gaining a place at the Royal Veterinary College to study Veterinary Medicine. Throughout her Animal Science degree Carrie enjoyed animal physiology and endocrinology, especially aspects related to reproduction. She is looking forward to using the knowledge and experience gained at Nottingham in her future career which she hopes will include work both in the UK and overseas.