# The Epididymis as a Target for Male Contraceptive Development

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**Abstract** The epididymis is an excellent target for the development of a male contraceptive. This is because the process of sperm maturation occurs in this organ; spermatozoa become motile and are able to recognise and fertilise an egg once they

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have traversed the epididymal duct. However, a number of attempts to interfere in sperm maturation and epididymal function or both have not been successful. The use of transgenic animals has proved useful in identifying a few epididymal targets but has yet to open the doors for drug development. Continuous focus on identifying additional epididymal targets and sperm-specific and epididymal-specific drugs is key to bringing a male contraceptive acting on the epididymis to the public.

**Keywords** Blood–epididymis barrier · Epididymal gene knockout mice · Epididymal proteins · Epididymal transporters · Epididymis · Sperm maturation · Spermatozoa

## 1 Introduction

Contraceptives acting by a post-testicular action in the male partner will be designed to take advantage of the physiology of the epididymis. Every spermatozoon entering the ejaculate has passed through this organ that promotes its transit from the testis, fosters its maturation and maintains its quiescence before ejaculation. As these processes take place over a period of about a week, there would appear to be ample time for the fertilising potential of spermatozoa to be compromised. If this could be accomplished, the onset of infertility would be rapid (the time it takes for the spermatozoa, in whatever region they are damaged, to complete epididymal transit and enter the ejaculate) and the infertility would be reversible and almost as fast (the time it takes the unaffected testicular spermatozoa to pass through the no-longer affected organ and replenish the caudal sperm reserves), i.e. about a week in both instances.

Various approaches to such epididymal contraception have been mooted. They are based on (1) promoting peritubular epididymal contractions, which would reduce sperm transit time so that the time for their interaction with epithelial secretions is reduced to a suboptimal level; (2) attacking epididymal epithelial secretion to modify the composition of luminal fluid so that concentrations of sperm maturation-dependent factors are reduced to a suboptimal level; and (3) directly targeting the spermatozoa with inhibitors of sperm function, for example, blocking sperm motility, metabolism, membrane function, vitality; however, none has been successfully implemented (Cooper and Yeung 1999). The challenge for investigators is to uncover potential epididymal targets for contraceptive development.

## 2 Infertile Males as a Contraceptive Paradigm

The disappointment arising from the difficulties in realising epididymal contraceptive leads has been countered by knowledge that several infertile males demonstrate precisely what is required of the concept of post-testicular contraception, and one

that occurs naturally or can be mimicked in transgenic animals. Such models provide hope that the ultimate goal is not illusory.

Several domestic species occasionally produce individual males that are sterile but are otherwise competent in other male (including copulatory) behaviour. Testicular function is normal, sperm numbers are not diminished but the ejaculates contain morphologically abnormal spermatozoa. The phenotype in these so-called "Dag defect" males is spermatozoa in ejaculates characterised by angulated flagella; they are motile but "swim backwards", that is, the head of the spermatozoon points away from the direction of motion. The origin of the flagella bending is the epididymis, since testicular spermatozoa from these males have straight flagella, but the site in the epididymis where coiling takes place differs among animals (Cooper and Barfield 2006). Male contraceptives could mimic this natural infertility if the causative mechanism were known.

Although tail coiling can be induced in spermatozoa by hypotonic treatments, the osmolality of cauda epididymidal fluid from the Dag defect bulls and boars was not consistently low and the osmolality of fluid from more proximal regions, where the effect may have originated, was not measured. Results of limited analysis of epididymal fluid composition were also inconsistent. The epididymal phenotype was not unusual and one pig examined had the initial segment (Cooper and Yeung 2003), a caput region that may be important in this condition in mice (see below).

## 3 Transgenic Mice: Epididymal Models of Male Infertility

Several murine models of male infertility display a similar angulated sperm defect to that of the Dag defect of domestic species. Tail angulation is normally present in a minority of cauda epididymidal spermatozoa when exposed to routine media (Eyden and Maisin 1978) but occurs to a far larger extent in certain knockout animals.

### *3.1 Infertile Male Mice Lacking the Initial Segment and Exhibiting Sperm Flagellar Angulation*

#### 3.1.1 c-Ros-Deficient Mice

The best characterised model of post-testicular infertility is the c-*ros* knockout mouse. Loss of this orphan tyrosine kinase receptor leads to male infertility, although the males are still capable of copulating and can be used instead of vasectomised mice to induce pseudopregnancy in female mice (Sonnenberg-Riethmacher et al. 1996). Post-copulatory spermatozoa in the uterus display flagellar angulation that prevents sperm migration beyond the uterotubal junction (Yeung et al. 2000).

Within the epididymis <20% of spermatozoa are angulated but this increases upon release from the cauda epididymidis in routine medium (Yeung et al. 1999). Flagellar angulation is a morphological manifestation of the swollen state (Yeung et al. 2002a) so that removal of the cell membrane with detergent membrane releases the membrane restraint and reduces the percentages of angulated and hairpin bend flagellar forms. Despite the infertility in vivo, c-*ros*-null spermatozoa are capable of fertilising zona-intact eggs in vitro (Sonnenberg-Riethmacher et al. 1996) so the null spermatozoa are capable of undergoing capacitation and the acrosome reaction, confirming that the in vivo infertility stems from a failure of the spermatozoa to reach the eggs as a result of their abnormal morphology.

In man, the c-*ros* gene is widespread along the epididymis, with the exception of the proximal caput epididymidis (Légaré and Sullivan 2004), rather than the high expression in the initial segment and lower in more distal caput segments. However, human male contraception will not involve gene knockouts; rather the mechanisms of infertility induction in these models – the induction of swelling – will be mimicked. The cause of the flagellar swelling in these animal models has been examined by analysing their epididymal pheno- and geno-types. The caput epididymidis of c-*ros*-null mice is smaller than that of the WT (Sonnenberg-Riethmacher et al. 1996) because it fails to develop the initial segment (IS) (Avram and Cooper 2004) with its associated rich vascularity (Wagenfeld et al. 2002). As expected, IS-specific genes are lacking, including CRES and MEP17 (Cooper et al. 2003), but also EAAC1, a sodium-dependent glutamate transporter, is down-regulated in the caput, but not corpus or cauda epididymidis (Wagenfeld et al. 2002). As a consequence, the glutamate content of cauda epididymidal spermatozoa is decreased (Yeung et al. 2004a). The significance of this lowered sperm osmolyte content becomes apparent when the epididymal spermatozoa contact fluids of lower osmolality at the time of ejaculation, and when the osmolytes are needed to remove water that enters osmotically.

At this moment c-*ros* appears to be a promising epididymal target for male contraceptive development. Its receptor and kinase domains are amenable to small molecular weight inhibitors and recently inhibitors of the c-*ros* kinase have been prepared (El-Deeb et al. 2009; Park et al. 2009). The challenge will be to examine the role of this gene in the adult male and determine whether regulating its expression will result in male infertility. If c-*ros* is not a druggable target, then potential downstream genes known to be regulated by c-*ros* are potential targets.

### 3.1.2 GPX5Tag2 Transgenic Mice

Deliberate targeting of the caput epididymidis with the large T-antigen, to interfere with its function, created two transgenic (TG) lines, one of which, the GPX5Tag2,

was infertile and displayed similar sperm flagellar angulation to that of the c-*ros*-null mouse. Unlike that in the c-*ros*-null-mutant, sperm angulation occurs, as in the Dag defect males, within the epididymis (Sipilä et al. 2002; Yeung et al. 2002b). Cauda epididymidal fluid osmolality is significantly lower in the TG male than that in the wild type, although still higher than that of the female tract (Sipilä et al. 2002). The initial segment is present, despite an apparent hypertrophy, and CRES and MEP17 are down-regulated (Sipilä et al. 2002), as found for the c-*ros*-KO males (Cooper et al. 2003). Unlike the angulated spermatozoa from the c-*ros*-KO males, those from GPX5Tag2 males are unaffected by demembranation and are unable to fertilise eggs in vitro. These results are explicable by the occurrence of hypo-osmotically driven flagellar bending within the epididymis, followed by the normal sulphydryl oxidation that occurs during epididymal transit and stiffens the flagellum into an angulated shape that cannot straighten out when membrane restraints are removed.

This animal model provides clues that changing the epididymal luminal fluid osmotic microenvironment will result in male infertility. The challenge will be to uncover molecules in the epididymis responsible for maintaining osmolarity and discovering approaches that interfere in the function of such molecules. These will include enzymes involved in the synthesis of *myo*-inositol and sorbitol and transporters for ions, glutamate, and L-carnitine.

## *3.2 Infertile Mice Lacking the Epididymal Initial Segment*

Many other models of murine male infertility exist, several of them presenting with angulated spermatozoa or lack of an initial segment but impaired volume regulation may not always be the cause of infertility. (1) The "*viable motheaten*" is an infertile male mouse with a natural mutation of the SH2 domain of the SHP-1 protein tyrosine phosphatase enzyme. This gene co-localises with, and dephosphorylates, c-*ros*; furthermore, the initial segment, as in the c-*ros*-KO, is lacking. The infertility, however, could also stem from testicular defects of spermatogenesis and testosterone secretion (Keilhack et al. 2001). (2) The epididymis of XX*Sry*, sex-reversed, pseudohermaphrodite males has no initial segment (LeBarr and Blecher 1986) and no rich capital vascularity (Le Barr and Blecher 1987) but is infertile because of azoospermia stemming from its abnormal chromosomal complement. The epididymal tubule is shorter in these males as the initial segment never develops (LeBarr et al. 1991), despite normal androgen levels (Le Barr et al. 1986). (3) The G protein-coupled receptor LGR4/GPR48 is expressed in the murine initial segment and the LGR4 knockout mouse is infertile and lacks an initial segment (Mendive et al. 2006; Hoshii et al. 2007). This is a consequence of early developmental changes leading to a hypoplastic organ together with down

regulation of the oestrogen receptor-α, aquaporin-1 and the sodium hydrogen exchanger NHE3 (*Slc9a3*). As a result, there is retention of spermatozoa and fluid within the testis, distension of the rete testis, leading to spermatogenic disruption, and sperm statis in the efferent ducts lumen with an immunological response in the form of granuloma (Mendive et al. 2006). Depending on the genetic background there may be angulation of spermatozoa in the cauda epididymidis (Hoshii et al. 2007).

It seems unlikely that these models will provide specific epididymal targets for contraceptive development. The SHP-1 protein is potentially attractive because it is a druggable target; however, the expression of this protein is ubiquitous and specificity is an issue. However, all models emphasise the importance of the proximal region of the epididymis in male fertility.

## *3.3 Infertile Mice with Angulated Spermatozoa*

### 3.3.1 Foxi1-Deficient Mice

The forkhead transcription factor *foxi1* regulates gene expression in narrow and clear cells of the epididymis, especially the vacuolar $H^+$-ATPase proton pump, carbonic anhydrase II and the chloride/bicarbonate transporter. As these proteins modulate the acidity of epididymal, luminal fluid pH is significantly higher in the KO than WT animals (Blomqvist et al. 2006), a feature also found in the c-*ros*-KO males (Yeung et al. 2004b). Sperm tail angulation is also a feature of these animals although the fact that spermatozoa fail to enter the uterus in large numbers suggests there are additional copulatory semen deposition problems. A change in epididymal morphology was also noted with a heavier cauda epididymidis present than that in wild type controls.

Although transcription factors are not normally considered to be druggable, their downstream targets could be possible targets for contraceptive development. Since *foxi1* regulates the expression of three druggable targets, i.e. two enzymes and one transporter, a male contraceptive could be designed to interfere in the function of either one or all three. It is not entirely clear whether all three or a combination of these targets would need to be compromised for male infertility. However, as lowered intraluminal pH is not invariably associated with male infertility (the ammonia transporter Rhcg KO mice have reduced intraluminal pH but are fertile: Biver et al. 2008), the other luminal fluid components could be targeted by contraceptives.

### 3.3.2 FKBP52-Deficient Mice

FKBP52 is a member of the family of immunophilins and also acts as a chaperone for steroid hormone receptors through Hsp90. Although FKBP52-null mice show partial androgen insensitivity in several reproductive tissues, e.g. external genitalia, the expression of androgen-dependent genes in the epididymis is normal (Cheung-Flynn et al. 2005; Hong et al. 2007). The infertile male phenotype observed in these mice is partially due to the disrupted external genitalia and anterior prostate, leading to poor mating and lack of vaginal plugs, but an epididymal defect has also been suggested (Hong et al. 2007). In the KO male, sperm numbers are decreased and sperm morphology is characterised by flagellar angulation in the cauda (but not caput or corpus), which would render males infertile were mating to be normal. FKBP52 has been shown to bind to spermatozoa, and spermatozoa from the null mice have abnormal morphology and a reduced fertilising ability. Nevertheless, in vitro capacitation and the acrosome reaction occur and fertilisation by these spermatozoa leads to normal embryo development (Hong et al. 2007).

Determining whether this protein is druggable warrants further study, for example, a drug could be designed to enter the epididymal lumen and prevent the interaction between FKBP52 and spermatozoa resulting in male infertility.

### 3.3.3 Herc4-Deficient Mice

E3 ubiquitin ligase (*Herc4*) is highly expressed in the testis and is involved in the flagging and removal of proteins during the spermatogenic sculpturing of spermatozoa. The knockout mice are subfertile (litter sizes reduced by half) and spermatozoa are less motile and display angulated spermatozoa (Rodriguez and Stewart 2007).

This is not a contraceptive model as infertility is not achieved.

### 3.3.4 SLO3-Deficient Mice

SLO3 (KSper) has been identified as a pH-dependent potassium channel involved in membrane hyperpolarization during capacitation. The KO males are infertile and their capacitated spermatozoa do not fertilise zona-intact or zona-free eggs *in vitro*. Up to 70% of capacitated spermatozoa exhibit flagellar angulation and display reduced progressive motility and a failure to undergo the acrosome reaction (Santi et al. 2010).

This is a promising lead as a contraceptive because it is a sperm-specific channel involved in a sperm-specific function.

### 3.4 *Infertile Male Mice with Flagellar Angulation Combined with Testicular Defects*

Other murine models that display angulated sperm defects may not purely reflect epididymal dysfunction; they may also be associated with testicular sperm defects. For example, mice deficient in (1) *ApoER2*, a member of the low density lipoprotein receptor family, which binds epididymal secretions of clusterin (Andersen et al. 2003) and *SePP1* (Olson et al. 2007), and is localised in the initial segment (Andersen et al. 2003). Spermatozoa from the Apolipoprotein E receptor 2 knockout mouse (ApoER-KO) display flagellar angulation that develops within the epididymis, but, unlike those of the GPX5Tag2 males, a large percentage of the spermatozoa can be straightened by detergent. Mitochondrial defects are also observed and PHGPX is reduced in the spermatozoa (Andersen et al. 2003). (2) Secreted hepatic selenoprotein P (*SePP1*) is central to selenium transport and SePP1-KO mice suffer neurological disorders. The null males are infertile with sperm flagellar abnormalities such as hairpin bends, which develop during epididymal transit, but extrusion of axonemes and outer dense fibres and a truncated mitochondrial sheath lacking several mitochondrial gyres is suggestive of testicular damage. Testicular Se is reduced in the *SePP1*-KO males (Renko et al. 2008) and supplementary Se does not reverse the sperm phenotype or infertility of the null males, as the carrier protein is absent (Olson et al. 2005). (3) Acid sphingomyelinase (*ASM*) catabolises sphingomyelin (SPM) to ceramide and phosphorylcholine. Human mutations in this gene (*SMPD1*) lead to lipid storage diseases (e.g. Niemann-Pick disease, NPD) in which SPM and associated lipids (e.g. cholesterol) accumulate in tissues. The *ASM*-KO mouse presents a pathological condition between NPD Types A and B and males suffer reproductive impairment (Butler et al. 2002). Flagellar angulation can be prevented by detergent treatment (indicative of osmotic swelling) and also by treatment with the lacking ASM (Butler et al. 2007), suggesting that membrane changes could also cause angulation.

The flagellar and axonemal defects described in these models point to inadequate spermatogenesis and spermiogenesis, rather than solely the inadequate epididymal function required for post-testicular contraception.

### *3.5 Infertile Male Mice Displaying Other Forms of Sperm Tail Angulation*

Several transgenic mouse models are characterised by males producing coiled sperm tails, but they are not the same sort of angulation as that mentioned above. (1) Spermatozoa from *Retinoid X receptor β-deficient* mice display tail angulation, but their infertility stems from oligoasthenozoospermia, as the testis is the main organ affected by lack of this receptor (Kastner et al. 1996). (2) *Spem1-deficient* mice display sperm tail bending that occurs at the sperm neck and reflects more a spermatogenetic failure related to failed cytoplasmic extrusion than an epididymal effect on volume regulation (Zheng et al. 2007). (3) Infertile *Gopc-(Golgi-associated PDZ- and coiled-coil motif-containing protein)-deficient* mice display flagellar coiling within the epididymis, the extent of which is related to migration of the cytoplasmic droplet (Suzuki-Toyota et al. 2004, 2007).

Mimicking the infertility of these males with testicular malfunction would not provide post-testicular contraception.

## 4 Targeting Other Epididymal Proteins

The importance of the initial segment for fertility may be shown by targeting other initial segment-specific secreted proteins. The genes of some proteins are apparently restricted to the initial segment, whereas others are also expressed in wild type animals in adjacent epithelial structures. In situ hybridization and immunohistochemical techniques have shown expression of genes and proteins limited to the IS, but without complete and serial sectioning of the caput epididymidis, the disposition of the medial and lateral aspects of the caput epididymidis (Blecher and Kirkeby 1978) makes boundaries determined in single sections incomplete. Molecular studies require dissection of tissue that cannot be done accurately when rapid freezing of the tissue is required. Nevertheless, there is some consensus of the regional expression of some proteins. The effect of the knockout of genes expressed in different epithelial structures may affect these epithelia directly but also have down- or up-stream effects on untargeted regions.

### *4.1 Infertility in Mice Involving Blockage of the Efferent Ducts*

#### 4.1.1 HE6-Deficient Mice

HE6, derived initially from the human epididymal caput (that largely contains efferent ducts: Yeung et al. 1991), encodes a G protein-coupled protein (Gpr64)

that is specific for the efferent ducts and the initial segment in rodents (Obermann et al. 2003). HE6-KO mice display reduced epididymal weight and sperm numbers, spermatozoa lacking heads and angulated flagella and reduced motility (Davies et al. 2004). This is a consequence of eventual blockage of the efferent ducts leading to dilation of the rete testis and spermatogenic arrest (Davies et al. 2004; Gottwald et al. 2006) and sperm stasis within the epididymis. Interestingly (and worthy of further investigation), and unlike the situation in the LGR4-KO males (Mendive et al. 2006), there is no immunological response. Gottwald et al. (2006) showed that water resorption in the efferent ducts was decreased in HE6-KO mice and spermatozoa accumulated within them so that a sperm-free epididymis resulted; results explained by the inability of the epididymis to cope with increased distal transport or further absorption of larger fluid volumes.

Unlike the LGR4-KO males, the initial segment is still present in these animals (Davies et al. 2004, 2007) and β-galactosidase is still expressed (Davies et al. 2004): gene expression is either decreased (cystatins 8 and 12, lipocalins 8 and 9, a novel β-defensin Defb42 and membrane protein HE9 [mE9], ADAM28, EAAC1) or increased (clusterin/ApoJ and osteopontin/Spp1) (Kirchhoff et al. 2006; Davies et al. 2007).

G protein-coupled proteins are excellent druggable targets, and that early spermatogenic stages persist in the testes of aged males and that there is a lack of immune response to the accumulated spermatozoa, raise the hopes of reversibility. Nevertheless, the longer the period of contraception, the more difficult it will be to clear the tract of the spermatozoa accumulated within the efferent ducts before resumption of fertility.

#### 4.1.2 Pax8-Deficient Mice

Pax8 is expressed in the efferent ducts and the initial segment. Thyroid-deficient Pax8-null mice can survive if given thyroxine, but the males are sterile. The null males are characterised by inconsistent development of parts of the epididymis and efferent ducts, whose presumed occlusion leads to dilatation of the rete testis and eventual spermatogenic shut-down (Wistuba et al. 2007).

The infertility here is related to azoospermia and not a post-testicular action.

### *4.2 Infertility After Targeting Epididymal Proteins*

#### 4.2.1 Immunological Depletion of P34H

Human epididymal protein P34H is secreted in the corpus epididymidis and binds to the spermatozoon over the acrosome and is involved in zona-binding (Boue et al.

1996); sperm levels are related to IVF success (Sullivan et al. 2006) and its loss can lead to human infertility (Boué and Sullivan 1996; Moskovtsev et al. 2007). It is the equivalent of P26h in the hamster in which immunological suppression leads to complete male infertility (Berubé and Sullivan 1994). P34H and related P31h are members of the carbonyl reductase family but whether they have this function in the epididymis remains to be examined.

This enzyme is a druggable target and if its activity were specific to the epididymis, it would be an ideal target for male contraceptive development.

#### 4.2.2 Immunological Depletion of Eppin

Eppin, an epididymal protease inhibitor (Wang et al. 2007), plays a role in post-ejaculatory seminal plug dissolution and release of physically arrested motile spermatozoa. Immunological suppression in monkeys leads to incomplete and irreversible infertility (O'Rand et al. 2006).

The risks of immunological contraception lie in the difficulty in ensuring adequate access of antibodies to spermatozoa within the epididymis (Nieschlag and Henke 2005). However, since Eppin is an enzyme inhibitor it is a druggable target.

### *4.3 Persistent Fertility After Targeting Epididymal Proteins*

For the proteins below, of initial segment origin or not, infertility has not been achieved in knockout models.

#### 4.3.1 SED1-Deficient Mice

SED1 (MFG-E8, lactadherin) is a protein that was identified as being involved in sperm–egg binding. It is secreted by the initial segment and then binds to the surface of the acrosomal region of sperm by intercalation of the discoidin/C domains. SED1 binds to the zona pellucida, but not to the egg plasma membrane (Ensslin and Shur 2003). Male SED1-null mice display a wide range of fertilities, from normal fertility to infertile with controls producing an average of 9 pups per litter compared with nulls that produce an average of 3 pups per litter (Ensslin and Shur 2003; see Shur et al. 2006 for review). Although sperm numbers, motility, morphology and rates of spontaneous and ionophore-induced acrosome reactions are normal, the spermatozoa display low sperm–zona binding.

SED1 could be targeted either in the epididymis or at the site of fertilisation for both male and female contraception.

### 4.3.2 SPAM1-Deficient Mice

SPAM1 (sperm adhesion protein 1, PH-20), a hyaluronidase, is present in the efferent ducts, initial segment, proximal epididymis, vas deferens and accessory organs (Zhang et al. 2004). It is secreted by the epididymis and is taken up on the sperm head, midpiece and tail during their transit in the epididymis (Martin-DeLeon 2006). SPAM1-KO male mice are fertile (Baba et al. 2002) possibly because of upregulation of other hyaluronidases (Hyalp1: Miller et al. 2007). The null-spermatozoa lack the protein but can take it up upon incubation in epididymal fluid. Upon uptake, these spermatozoa can be capacitated and penetrate oocyte-cumulus complexes as well as wild-type spermatozoa. Even wild type spermatozoa can take up SPAM1 from epididymal fluid, suggesting an undersaturation (Chen et al. 2006) that may have a physiological consequence, since SPAM1 is also secreted by the uterus, binds to spermatozoa and enhances cumulus dispersal (Griffiths et al. 2008a). The uptake of both the epididymal and uterine forms of SPAM1 is mediated by epididymosomes and uterosomes (Griffiths et al. 2008b).

As an enzyme, SPAM1 is druggable, but has yet to be shown to be targetable.

### 4.3.3 CRISP1-Deficient Mice

CRISP1 (the former protein DE, *c*ysteine-*ri*ch *s*ecretory *p*rotein) is secreted beyond the initial segment binds to spermatozoa and is involved in sperm–egg fusion (Ellerman et al. 2006; Roberts et al. 2006). However, CRISP1 knockout male mice are fertile because the number of spermatozoa, the motility of fresh and capacitated spermatozoa and their morphology are normal (Da Ros et al. 2008). Although the extent of tyrosine phosphorylation is below that of control spermatozoa, their ability to undergo progesterone-induced acrosome reactions is unchanged from that of WT controls. In vitro fertilisation reveals a lowered propensity to fertilise both zona-intact and zona-free eggs; furthermore, the fusion ability of Crisp1-KO spermatozoa is reduced by addition of Crisp1 and Crisp2 during gamete co-incubation. This raises the possibility that Crisp2 may be upregulated in the Crisp1-KO mouse, explaining the fertility of these animals.

The druggable signature of this protein and its interaction with spermatozoa remains to be determined, but the fertility of the KO males is discouraging.

## *4.4 Infertility in Mice Involving Blockage of the Distal Duct*

### 4.4.1 Juvenile Steatosis

Defects in the carnitine transporter OCTN2 (*slc22a5*) lead to primary carnitine deficiency in mice. At 8–9 weeks of age the epididymis becomes deformed with a greater weight than that of the WT as the proximal duct becomes dilated with accumulated spermatozoa. As these are extravasated into the stroma, immune responses follow, leaving the distal duct void of spermatozoa and the males infertile because of azoospermia (Toshimori et al. 1999). In the mutant males, the carnitine transporter is found on the apical side of the epididymal epithelium distal to the site of sperm accumulation (Yakushiji et al. 2006).

Interference in the function of L-carnitine in the epididymis has been a challenge because it has been difficult to deplete completely the normal very high intraluminal concentrations in the epididymis (Hinton et al. 1979). Chemical depletion does not lead to male infertility in rats (Cooper et al. 1997) or hamsters (Lewin et al. 1997). Further, L-carnitine plays a major role in lipid metabolism in many other tissues and specificity maybe an issue. However, several L-carnitine transporters have been identified (Tamai et al. 2000; Eraly et al. 2004; Koepsell et al. 2007) and although some have overlapping tissue expression, some may be unique to the male reproductive tract.

Organic transporters such as *slc22a5* are druggable targets and are also excellent vehicles for transporting potential contraceptives into the epididymis (see below).

### 4.4.2 RARα-Deficient Mice

The males of retinoic acid receptor-α-KO mice are either infertile or have reduced fertility, as a consequence of the epithelia lining the ducts of the epididymis and vas deferens exhibiting squamous metaplasia. Although spermatozoa develop normally in the testis, they degenerate in the epididymis and vas deferens because inspissated ductal fluid blocks the normal passage of the spermatozoa (Costa et al. 1997).

These models of highly disturbed epithelial function are not useful as contraceptive paradigms, since inspissation of spermatozoa and immunological sequelae make any contraception irreversible.

# 5 The Blood–Epididymis Barrier as a Hurdle and an Opening to the Administration of Putative Male Contraceptives

## *5.1 A Physical Barrier*

The blood–epididymis barrier comprises more than just tight junctions. Cell–cell contacts such as tight junctions found in many epithelia are effective in preventing the passage of molecules from entering into a lumen or other specialised compartments. Tight junctions between epididymal epithelial cells are no exception and Friend and Gilula (1972) wrote; "Among the various epithelial cell contacts examined, the zonula occludens of the epididymis is the most highly developed". Later studies by Suzuki and Nagano (1978) and more recently by Cyr and colleagues (Gregory and Cyr 2006; Dubé et al. 2007; Cyr et al. 2007) have shown the extensive and complex nature of these junctions. As one might expect, the classic tracer lanthanum does not pass between the tight junctions if the tracer is injected into animals (Hoffer and Hinton 1984). Therefore, the tight junctions form a formidable hurdle for putative male contraceptives entering the epididymal lumen to affect the maturing spermatozoa.

The permeability of the tight junctions (paracellular pathway) has been extensively studied using micropuncture studies (Hinton and Howards 1981, 1982; Turner et al. 1981; Yamamoto and Turner 1990) and low molecular weight molecules such as water and urea pass into the lumen readily from blood. Any molecule larger than 160 kDa fails to enter, or only a very small amount enters, the lumen. At first glance, these findings would suggest that it would be challenging to identify a putative low molecular weight male contraceptive compound that would readily enter the epididymal lumen at high enough concentrations to affect the maturing spermatozoa. The answer to this dilemma is that the blood–epididymis barrier comprises more than just tight junctions.

## *5.2 A Physiological Barrier*

The blood–epididymis barrier can also be considered to be a physiological barrier and clues to this originated from some of the micropuncture studies described above. If a non-metabolizable form of glucose, L-glucose, is injected into blood, it does not readily enter the lumen of the epididymis. If a non-metabolizable form of glucose, 3-*O*-methyl-D-glucose, the D-isomer, is injected, it is readily transported into the epididymal lumen (Hinton and Howards 1981). This would suggest that the blood–epididymis barrier is not an absolute, but a restrictive barrier, in that it only allows certain molecules to enter into its cells and lumen. The restrictive nature of the blood–epididymis barrier is a reflection of the permeability properties of the basolateral and apical membranes, which in turn reflect the transporting properties of various transporters and channels within them.

Therefore, transporters can either be targets themselves for contraceptive development or their transporting or permeability properties can be used to move contraceptive agents into the epididymis. An example of the latter, albeit for the testis, is when doxorubicin (adriamycin), an entineoplastic drug used for the treatment of many cancers, is administered to males an infertility phenotype is observed. This was shown to be due to germ cell decline in phospholipids and subsequent germ cell loss from the epithelium (Meistrich et al. 1985; Zanetti et al. 2007). Later studies showed that the transporter *slc22a16* (CT1; Enomoto et al. 2002), an organic cation transporter that transports L-carnitine, was identified as the primary candidate regulating the influx of doxorubicin (Okabe et al. 2005). The transporter *slc22a16* is highly expressed in the testis and to a lesser degree in the human epididymis (Enomoto et al. 2002). Therefore, the transporting properties of epididymal organic solute transporters could be exploited in a similar manner.

### *5.3 Epithelial Transporters as Targets or Vehicles for Male Contraceptive Development*

Several transporters have been identified in the epididymis from gene microarray results (Jervis and Robaire 2001; Cornwall and Hann 1995; Cooper et al. 2004; Johnston et al. 2005; Jelinsky et al. 2007) but very few have been studied to any significant degree. The most studied series of transporters in the epididymis are the ion and water transporters (see reviews by Leung et al. 2004; Pastor-Soler et al. 2005) and a clue to their importance in male fertility came from the *Foxi1*-null mutation described earlier. Transporters are excellent targets for male contraceptive development because they are amenable to small molecular weight inhibitors and some, for example, those located on the basolateral membrane, e.g. OCTN2 (Rodríguez et al. 2002), are easily accessible to inhibitors present in the blood. Several inhibitors have already been designed that interfere with organic solute transporter activity and such inhibitors have proven useful in the clinic (Sweet et al. 2001; Ohtsuki 2004; Sai and Tsuji 2004; El Elwi et al. 2006; Koepsell et al. 2007).

## 6 Conclusion

Despite the many transgenic models reviewed above that are associated with male-selective fertility impairment, not all can serve as paradigms for post-testicular contraceptive development. Some do not bring consistent infertility whereas others are associated with spermatogenic damage. The combination of epididymal epithelial defects and sperm angulation inherent in c-*ros*-KO males is not echoed in all the transgenic models: flagellar angulation is more associated with infertility than a morphological expression of epididymal abnormality, although anatomically

invisible physiological deficiencies in the epididymal epithelium may well underlie the susceptibility of the sperm tail towards angulation.

Current knowledge on the role of epididymal osmolytes in sperm volume regulation suggests targets that could induce angulated spermatozoa: channels or transporters involved in the transport, uptake and efflux of osmolytes by the epididymis and spermatozoa. They all need to be characterised and their modes of regulation determined; in some areas, a start has been made, in others research needs to be initiated. To cover the possibility that epididymal- and sperm-specific drugs are not found, research on targeting of drugs to the epididymis needs to be started. In this regard, some epithelial channels involved in osmolyte provision could be hijacked for surreptitious entry of inhibitors into the epididymal lumen.

Research into factors affecting the initial segment, regulators of epithelial channels and transporters and inhibitors of sperm osmolyte influx and efflux should proceed together so as to be able to target inhibitors of sperm function to the epididymal lumen.

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