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7

Androgen receptor roles in spermatogenesis and infertility

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Androgens such as testosterone are steroid hormones essential for normal male reproductive development and function. Mutations of androgen receptors (AR) are often found in patients with disorders of male reproductive development, and milder mutations may be responsible for some cases of male infertility. Androgens exert their action through AR and its signalling in the testis is essential for spermatogenesis. AR is not expressed in the developing germ cell lineage so is thought to exert its effects through testicular Sertoli and peri-tubular myoid (PTM) cells. AR signalling in spermatogenesis has been investigated in rodent models where testosterone levels are chemically supressed or models with transgenic disruption of AR. These models have pinpointed the steps of spermatogenesis that require AR signalling, specifically maintenance of spermatogonial numbers, blood-testis barrier integrity, completion of meiosis, adhesion of spermatids and spermiation, together these studies detail the essential nature of androgens in the promotion of male fertility.

Introduction

The mammalian testis has two functions: spermatogenesis (the production of haploid germ cells) and steroidogenesis (the production of the steroid hormones that support male reproductive

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development and function). Disruption of testosterone production by hypophysectomy [1], Leydig cell ablation [2] or knockout of luteinising hormone receptor [3] demonstrate that spermatogenesis cannot proceed to completion without testosterone.

The receptor for testosterone is the steroid nuclear hormone receptor androgen receptor (AR, OMIM entry: 313700). *Ar* is a single copy gene on the X chromosome [4]. The classical genomic mechanism of testosterone signalling occurs when testosterone diffuses into the cell and binds to AR, then the ligand–receptor complex translocates to the nucleus and where it binds to androgen response elements (AREs) in the regulatory regions of genes to modify their translation. Non-classical signalling occurs when the ligand–receptor complex or testosterone itself activates non-genomic cytoplasmic signalling pathways, or when in certain circumstances the AR binds to AREs in the absence of testosterone [reviewed in [5,6]].

Despite requiring androgens for their survival and maturation, germ cells do not express androgen receptors and germ cell-specific androgen receptor expression is not required for their normal maturation [7–9]. However, the somatic Sertoli, PTM, Leydig, vascular endothelial and vascular smooth muscle cells of the mature testis express androgen receptor [10], and it is widely accepted that the requirement of testosterone for spermatogenesis is mediated by these cell types.

Rodent models of androgen signalling disruption

Global androgen receptor knockout models

The first animal model of androgen insensitivity was described in 1970 by Lyon and Hawkes, who reported an X-linked gene for 'testicular feminisation' (*Tfm)* in the mouse [11]. It was subsequently discovered that male *Tfm* mice carry a single nucleotide deletion in exon 1 of their *Ar* gene, the resulting frameshift introduces a premature termination codon [12]. With the advent of Cre-*loxP* technology [reviewed in [13]], mice with a total androgen receptor knock-out (ARKO) genotype have been produced by four groups. By mating a mouse line with floxed region of the *Ar* gene to one expressing Cre recombinase under the control of a constitutively active gene, genetic ablation of part of the AR resulting in loss of functional protein was achieved in all cells of the resulting offspring [14–17]. Both ARKO and *Tfm* mice have small, inguinal testes, lack of Wolffian duct structures and feminisation of external genitalia. When examined histologically, some tubules in the testis lack germ cells completely and the others contain a few spermatogonia, but no post-meiotic germ cells. Normal spermatogenesis does not occur. This is analogous to testicular histology seen in CAIS patients [17,18].

Cell specific androgen receptor ablation models

Models of testosterone or androgen receptor disruption can demonstrate what processes in the testis rely on AR signalling, but they cannot determine which cell type(s) mediate these processes. Recent studies of cell-specific ablation or overexpression of AR in the testis using the Cre-*loxP* system have elucidated both the cell and AR-specific actions of androgens in the testis [19]. Mouse lines with cell-specific knock-outs of AR in the male reproductive system are detailed in Table 1. Through these studies it has become apparent that AR signalling in the PTM and Sertoli cells exerts the most significant effects on spermatogenesis.

Sertoli cells are mesoepithelial somatic cells that coordinate and structurally support the maturing germ cells. Each Sertoli cells spans the seminiferous tubule from the basement membrane to the tubule lumen, and is tightly linked to a set of germ cells at specific stages by different types of junction protein complexes [20]. Sertoli cells have a large surface area with a well-developed cytoskeleton that assists in maintaining their shape as well as providing a scaffold for the movement of germ cells [21,22]. Since germ cells do not express AR it is likely that the action of androgens on spermatogenesis is potentiated through their binding to Sertoli cell AR. Androgen receptors are not present in the Sertoli cells of the mouse until postnatal day 4 when faint staining of occasional Sertoli cells is seen. Staining of all nuclei and a progressive increase in staining intensity is noted from day 5 [23]. After maturation, the expression of AR in Sertoli cells fluctuates in a seminiferous tubule stage-specific manner [10], appearing strongest at stages VI–VII. In humans, Sertoli cell AR has been reported as either weakly

Table 1
Summary of Cre lines used for cell specific ablation of AR action in the male reproductive system.

Cell type	Promoter	Effect on mouse fertility	Reference
Sertoli	*Amh*-Cre	Infertile. No post-meiotic germ cells.	[14,29,34]
Peri-tubular myoid	*Myh6*-Cre	Infertile. Reduction in germ cells at all stages.	[41]
Leydig	*Fabp4*-Cre	Fertile, but degeneration of seminiferous epithelium occurs with ageing. Leydig cells do not mature correctly.	[76]
Spermatocytes	*Sycp1*-Cre	Fertile	[7]
Vascular smooth muscle	*Tagln*-Cre	Fertile but testicular vasomotion impaired	[77]
Vascular endothelial	*Tie2*-Cre	Fertile	[78]
Prostate epithelium	*Arrpb2*-Cre	Fertile	[79]
Prostate, vas deferens and epididymal epithelium, SV smooth muscle	*Arrpb2*-Cre	Subfertile, defects in epididymal transit of sperm	[80]
Prostate smooth muscle	*Myh6*-Cre	Phenotype complicated by loss of AR in PTM.	[81]
SV smooth muscle	*Myh6*-Cre	Phenotype complicated by loss of AR in PTM.	[82]
Neuronal	*Nes*-Cre	Subfertile, variable phenotype with fewer litters and pups per litter. Altered mating behaviour.	[83,84]
Epididymal epithelium	*Foxg1*-Cre; *Rnase10*-Cre	Infertile due to obstructive azoospermia.	[85,86]

detected or not present up to 5 years, but is detected from 8 years onwards and is strongly expressed in adulthood [24–26]. Expression is also cyclical in men, appearing strongest at stage III [27]. A model of androgen receptor overexpression in Sertoli cells (tgSCAR) from day 2 onwards results in premature maturation of Sertoli cells, reduced final Sertoli cell number and therefore a reduced final germ cell number [28], suggesting it has a key role in Sertoli cell maturation. Development of tgSCAR germ cells was accelerated corresponding to premature maturation of the Sertoli cells, illustrating the intimate dependence of germ cells on Sertoli cell androgen receptor signalling for their coordinated development.

Sertoli-cell specific AR knockout (SCARKO) mouse lines have been created by two groups using an inserted Cre recombinase coding sequence driven by an anti-Müllerian hormone promoter [14,29]. The testes of SCARKO mice develop normally with an external phenotype similar to their control male littermates, with fully descended testes and intact Wolffian duct-derived structures, unlike the ARKO model where they are absent [17]. However at post-natal day 50 their testes are approximately a third of the weight of their wild type littermates' and contain few post-meiotic germ cells more advanced than pachytene primary spermatocytes, indicating a meiotic block. SCARKO seminiferous tubule diameters are decreased and the tubules do not develop a normal lumen. It is important to note that mice with a Sertoli cell-specific ablation of the second zinc finger domain (vital for DNA binding) display a similar post-meiotic block phenotype, indicating that progression of spermatocytes through meiosis is an event dependent on classical genomic signalling of androgens [30]. The two groups differed in analysis of the levels of testosterone and LH in their SCARKO models; where De Gendt and colleagues found these two hormones to be normal in adult mice, the Chang group found LH to be high and testosterone to be low. Spermatogonia [14] and Sertoli cell numbers do not differ between SCARKOs and controls [31]. There is controversy as to whether Leydig cell numbers in the SCARKO are maintained [32] or are decreased [33] which would imply that Sertoli cell AR activates a paracrine signalling pathway that affects Leydig cell division. Another study created a 'hypomorphic' SCARKO with Sertoli cell-specific reduction of AR action, but also a general reduction in levels of AR protein throughout the whole body due to a phenotypic effect of the placing of the *loxP* sites in the transgenic AR [34].

Seminiferous tubules are surrounded by a layer of peri-tubular myoid (PTM) cells. PTM cells are contractile and produce peristaltic waves in the seminiferous tubules that propel mature spermatozoa towards the rete testis [35]. PTM cells synthesise the basement membrane components laminin, collagen and fibronectin in co-operation with Sertoli cells [36,37]. Like other tissues in the reproductive system a reciprocal relationship exists between the mesenchymal (PTM) and epithelial (Sertoli) cells of the testis, which is vital for the maintenance and function of both cell types. The mesenchymal PTM cells are the first cell in the testis to express AR: at or before e16.5 in the mouse [38] and at e18.5 in the

rat [39,40]. Cell-specific ablation of AR in the PTM cells has been achieved using Cre/*loxP* transgenic mice (PTM-ARKO) [41]. PTM-ARKO testes are 70% smaller than controls because of a reduction in numbers of all germ cell stages.

Even though these two particular cell types appear to be the most important for transmission of androgen signalling to developing germ cells, AR is present in many different cell types in the male reproductive system that may not have an immediate impact on spermatogenesis but still contribute to fertility and normal testicular function, as well as is in other non-reproductive organs throughout the body. Conditional AR ablation lines have been created and investigated to elucidate its role in these cell types [42]. Table 1 summarises conditional ablation of AR in several different cell types of the male reproductive system and whether this ablation has any impact on spermatogenesis, testicular function or wider fertility. The results of these cell ablation experiments draws attention to the absolute requirement for AR signalling to maintain overall synergy and function of the male reproductive system, which contributes to overall male fertility.

Models with decreased testosterone production

Before the Cre-*loxP* system was widely used to interrogate cell specific AR action, changes in spermatogenesis were identified in rodent models with reduced testosterone production. Mice with a luteinising hormone receptor knockout (LuRKO) produce levels of testosterone 10 to 20 times lower than wild-type [3]. Another model is the 'TE' rat that carries implants containing low-dose testosterone and estradiol to suppress LH production at the pituitary and therefore reduce testicular T levels to 3% of control [43]. Although reduction in AR signalling in these models occurs in all cell types that express the receptor and therefore the resulting phenotype cannot be assigned to lack of AR action in a specific cell type, they have provided a number of novel observations that add to the complete picture of AR signalling in spermatogenesis.

Control of spermatogenesis by androgens

Experimental perturbation of testosterone and/or androgen receptor has highlighted specific steps in spermatogenesis that require AR signalling, summarised in Fig. 1 and described here.

Maintenance of spermatogonia numbers

PTM-ARKO mice have a reduction of germ cell numbers at all stages, (including spermatogonia) that is not seen in any other conditional ARKO model [41], suggesting that maintenance of spermatogonia numbers is controlled by PTM cell AR. Since spermatogonia are juxtaposed to the basement membrane they are in intimate contact with the PTM cells. It is possible that this affect is due to direct signalling between PTM cells and spermatogonia. Since PTM cells in the PTM-ARKO also demonstrate a progressive loss of desmin and smooth muscle actin (SMA) it is likely that loss of AR is affecting the smooth muscle phenotype in PTM cells, and a disruption of laminin indicates problems with the basement membrane of the seminiferous tubules. These disruptions may impair attachment and signalling between PTM cells and spermatogonia and thus disrupt their niche [44]. It is also possible that the decrease in spermatogonia in the PTM-ARKO is mediated through changes in Sertoli cells. The decrease in germ cells is not due to a decrease in Sertoli cell number, as this is not reduced in PTM-ARKO mice. However, levels of Sertoli cell-specific gene products including *Rhox5*, *Epp* and *Tubb3* are reduced in PTM-ARKO testes, implying that AR signalling in PTM cells has an effect on the transcriptome and function of their neighbouring Sertoli cells. *In vitro* evidence has demonstrated that androgens can stimulate PTM cells to secrete a factor or factors dubbed "P-mod-S" [45] that modulates Sertoli cell function, but it remains as yet unidentified, and its role is disputed by some investigators [46].

Progression through meiosis

Very few post-meiotic cells are noted in the SCARKO mouse. Morphological analysis of SCARKO testes established that germ cell entry into meiosis appeared normal, but a progressive loss of

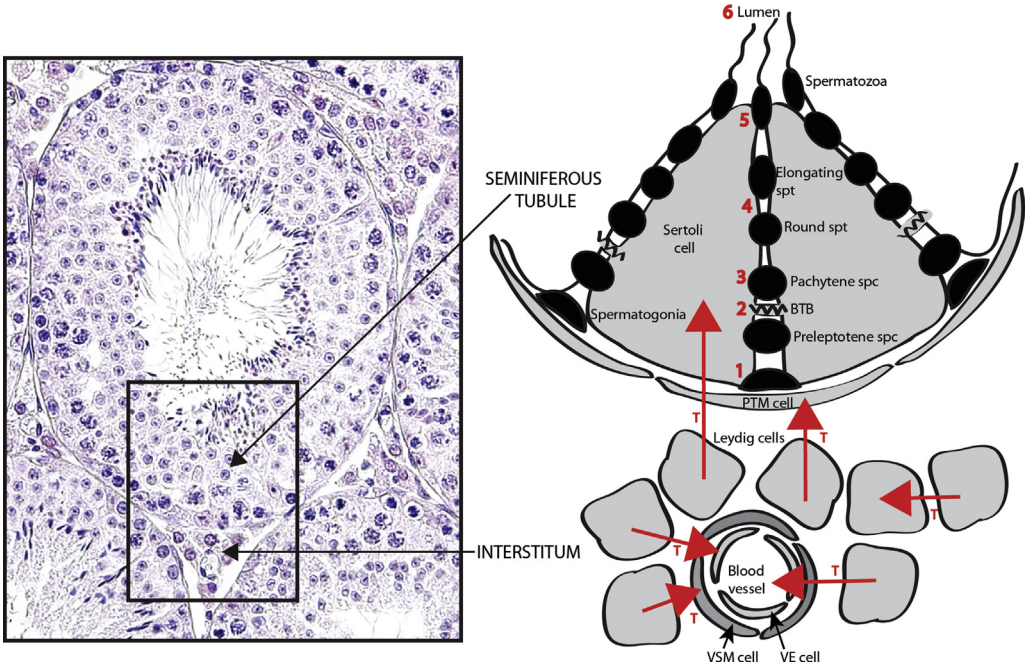

Fig. 1. The anatomy of the testis and control of spermatogenesis by androgens. The testis consists of seminiferous tubules surrounded by an interstitial stroma. The stroma contains steroidogenic Leydig cells and blood vessels that are lined by vascular endothelial (VE) and vascular smooth muscle (VSM) cells. Peritubular myoid (PTM) cells line the outside of the seminiferous tubule. Sertoli cells extend from the PTM cells to the lumen and support and contact the developing germ cells. Junctions between Sertoli cells form the blood testis barrier (BTB). Maturation of germ cells in spermatogenesis progresses toward the lumen starting with the undifferentiated diploid spermatogonia on the basement membrane to spermatozoa that are released from the apical surface of the Sertoli cell. During meiosis, spermatocytes transit the BTB. Testosterone produced by the Leydig cells has autocrine effects on the Leydig cells themselves, which express AR, as well as paracrine effects on VE, VSM, PTM and Sertoli cells of the testis. It also diffuses into the interstital blood vessels to be transported to the circulatory system. There are five critical processes in spermatogenesis that are regulated by testosterone labelled 1–5 on the figure. (1) Maintenance of spermatogonial numbers, (2) maintenance of the BTB, (3) completion of meiosis by spermatocytes, (4) adherence of elongated spermatids to Sertoli cells, (5) the release of mature spermatozoa and (6) the formation of the seminiferous tubule lumen.

pachytene primary spermatocytes between stages VI and XII was noted. Round spermatid number was 3% of controls and no elongating spermatids were seen [14]. In rodent models that have reduced testosterone production such as the LuRKO mouse and TE implanted rats, meiosis completes and spermatogenesis proceeds to the round spermatid stage implying that meiosis itself is not sensitive to low testosterone levels, even though subsequent stages may be. Ablation of any residual androgen action in the LuRKO mouse with flutamide (an AR antagonist) results in the reversion of the phenotype to a meiotic block which adds to the evidence that at least a low level of AR signalling is required for completion of meiosis [3].

BTB integrity

Tight junctions between adjacent Sertoli cells above the level of leptotene spermatocytes form the blood-testis barrier (BTB), a structure that partitions the adluminal compartment of the seminiferous tubule from the basal compartment so haploid germ cells can mature in an immunologically privileged site with a specialised microenvironment [47]. During maturation, sperm move along the Sertoli cell, traversing the BTB during meiosis. It is accepted that androgen signalling contributes to the maintenance of the BTB, but some studies suggest that it is essential and that the BTB is not present [48,49], whereas others suggest that tight junctions in the SCARKO can form, but BTB formation is delayed and

incomplete [50]. A number of genes encoding BTB proteins are down-regulated or incorrectly expressed in SCARKO mice [49,51,52]. Because of the intimate association between barrier formation and meiotic progression, it has been postulated that the two processes are co-dependent, but more studies on barrier formation in other mouse models with a meiotic block is required to confirm this hypothesis [50].

Seminiferous tubule lumen formation

Sertoli cells secrete the seminiferous tubule fluid that forms the tubule lumen and acts as a vehicle to transport mature spermatozoa from the testis. It contains appropriate nutritional and hormonal factors necessary to support spermatogenesis that are unavailable from the circulation due to the presence of the BTB. The formation of the seminiferous tubule lumen depends on Sertoli cell androgen receptor as SCARKO testes do not have functional tubal lumens [14]. When efferent ducts are ligated, control testes increase in weight but SCARKO testes do not, implying that Sertoli cell fluid secretion is affected when AR is ablated [50]. It has been suggested that meiosis can only be completed after Sertoli cell fluid secretion has been established [reviewed in [53]], and also that a lumen cannot be formed when the BTB is disrupted [54]. There appears to be a functional link between the BTB, progression of meiosis and secretion of seminiferous tubule fluid by Sertoli cells that requires further elucidation.

Spermatid adhesion

Because of the presence of the post-meiotic block in SCARKO mice it is impossible to use this model to pinpoint the contribution of Sertoli cell AR signalling to subsequent stages of spermatogenesis, so systemic models of androgen or androgen receptor disruption have been used instead. 'TE implanted' rats and a hypomorphic AR mouse model have both been used to determine that both Sertoli-spermatid adhesion and spermiation are dependent on androgens. A reduction in testosterone results in premature detachment of spermatids from Sertoli cells at the round to elongating transition stage. Large numbers of round spermatids accumulate in cauda epididymis in both TE implanted [55] and hypomorphic AR mice [34]. The round to elongating spermatid transition stage is also when desmosome-based connections that attach spermatids to their supporting Sertoli cell are replaced by ectoplasmic specialisations (ES), a type of adherens junction unique to the testis, promoting the theory that ES may be disrupted in models with low testosterone signalling. However, normal ES were noted in TE rats when testes were examined by electron microscopy [56].

Spermiation

Both the TE rat and hypomorphic mouse models also note spermiation failure characterised by elongating spermatid retention with subsequent phagocytosis by Sertoli cells [34,57]. Release of the sperm from the seminiferous epithelium during spermiation involves the replacement of the apical ES by tubulobulbar complexes (TBC), structures thought to internalise disassembled apical ES junctions in preparation for the release of spermatids [58]. TBC formation and removal of ES are not affected by hormone withdrawal, but final disengagement of the spermatid from the Sertoli cell is affected [59]. Further investigation revealed that androgen action is necessary for the disengagement of a Sertoli cell complex containing $\alpha 6\beta 1$-integrin and phosphorylated focal adhesion kinase (FAK) [60] from laminin $\alpha 3\beta 3\gamma 3$ on spermatids [61].

Control of both of spermatid adhesion and spermiation has been shown to be through the non-classical AR signalling pathway *via* action of Src kinase. Sertoli cells in culture expressing a mutated form of AR that can't activate Src bind fewer germ cells, and explants treated with a Src inhibitor release fewer sperm [62]. Three days after *in vivo* injection of a Src inhibitor, spermatocytes and round spermatids were absent but elongating spermatids remained [63]. It is interesting to note that AR action appears to be required for both the attachment and disengagement of maturing germ cells at different stages of spermatogenesis. Since the nature of the junctions that hold germ cells to Sertoli cells is in flux throughout spermatogenesis, it may be that Src has different roles in each of these different junction

complexes that are yet to be fully characterised. Although defects in spermatid adhesion and spermiation cannot be definitively assigned to AR signalling in the Sertoli cell (because AR signalling is reduced in all testicular cell types in these models), it is likely that the Sertoli cell is responsible rather than another testicular cell type due to the isolated environment in which post-meiotic germ cells develop behind the BTB. Studying BTB remodelling, spermatid adhesion and spermiation as a coordinated process rather than isolated events may help further elucidate the androgen dependent coordination of spermatogenesis. There is already evidence that fragments of laminin chains produced during spermiation induce remodelling of the BTB, thus intimately coordinating these two androgen-dependent steps in the processes of spermatogenesis [64].

Clinically relevant mutations of androgen receptor

Mutations that result in AIS

As of October 2014, more than 1100 mutations in human *AR* have been identified [65]. Although some *AR* mutations are associated with non-testicular phenotypes such as prostate cancer, premature ovarian failure and Kennedy's disease, approximately 90% result in androgen insensitivity syndrome (AIS). AIS presents as a spectrum of masculinisation disorders in XY individuals from a fully female external phenotype present in complete androgen insensitivity syndrome (CAIS) through undervirilised male phenotype in partial androgen insensitivity syndrome (PAIS) to normal male genitals but infertility seen in mild androgen insensitivity syndrome (MAIS). PAIS and CAIS are discussed further in the chapter on androgen insensitivity syndrome by Mongan et al., in this issue of *Best Practice and Research Clinical Endocrinology and Metabolism.*

In contrast to more severe forms of AIS, patients with MAIS have normal genitalia and may present with infertility as the first or only symptom. Investigation into *Ar* mutations should be prompted when a high androgen sensitivity index (ASI, the LH to testosterone ratio) is seen as there is a positive correlation between the two [66]. The location of the mutation in the AR gene and the type of the mutation correlates with the resulting severity of the AIS phenotype. Most premature termination mutations result in a truncated AR protein and complete AIS. Single base substitutions can results in all grades of AIS, depending on what effect the substitution has on the amino acid sequence and three dimensional structure of the protein and therefore how much of the function is retained. Exon 1 of *AR* encodes the N-terminal domain which codes for more than half the AR protein, but only about 25% of total loss of function mutations occur in this domain. While mutations in exon 1 such as premature termination mutations or deletions generally result in CAIS due to a truncation of the protein, 22 of 39 of the single-base substitution mutations in exon 1 that result in a form of AIS results in MAIS, implying that missense mutations in this domain have a mild effect on AR function. Since the main role of the N-terminal domain is as a binding site for other transactivation proteins, mutations may impair the binding or activity of these factors and result in a protein that may have reduced efficiency in specific cell types only. For example, in one patient, the mutation prevented interaction of the AR with a TIF2, Sertoli-cell specific co-activator, thus impeding the genomic actions of AR only in Sertoli cells [67]. The frequency of *AR* mutations in patients with oligospermia or azoospermia under investigation for infertility has been shown to be 2–3%, a relatively small proportion of infertile men [66]. However mutations of genes up or downstream of AR in its signalling pathway may also be contributing to cases of infertility.

Association of AR trinucleotide repeats with infertility

Longer variants of a CAG polyglutamine (Q tract) and a GGN polyglycine (G tract) trinucleotide repeat in exon 1 of *AR* have been suggested to have an association with infertility. AR transactivation [68] and levels of AR protein and mRNA [69] have been shown to be reduced *in vitro* by increasing CAG repeat number and longer polyglutamine tracts are associated with impaired sperm production in men [70]. Despite this, subsequent studies have been conflicting about the association [reviewed in [71]]. Further downstream of the polyglutamine tract is a polyglycine GGN tract (G tract) which varies in length from 10 to 30 repeats. Deletion of the polyglycine tract resulted in a 30% reduction in

transactivation potential *in vitro* [68]. However, there is also conflicting evidence about whether the number of GGN repeats has an association with male infertility.

Summary

Testosterone signalling through AR is vital for complete spermatogenesis and this is mediated by the somatic cell types of the testis that express specifically the Sertoli and PTM cells that have intimate connections with the maturing germ cells (summarised in Fig. 1). Although the points at which androgen signalling affects spermatogenesis *via* PTM cells and Sertoli cells are well characterised, the effectors of this paracrine signalling network are not yet fully elucidated. Array studies on the SCARKO model have produced data on gene expression changes [reviewed in [63]], but have identified surprisingly few for a model with such a complex phenotype. More recently, immuno-precipitation of actively translated mRNAs has identified novel androgen-regulated Sertoli cell transcripts [72] and a population of microRNAs have also been shown to change testosterone deprivation model [73] and these data will be useful as starting points for future studies. Unravelling the combination of classical AR signalling, non-classical AR signalling and microRNA regulation in both Sertoli and PTM cells is a significant task for future research that must now focus on downstream targets of testosterone signalling if we are to fully understand and clinically support spermatogenesis in patients, an increasing number of whom are presenting with low sperm count [74,75].

Research agenda

- The advent of next generation sequencing will contribute to the need for personalised genomic medicine.
- Further research into the fundamental mechanisms of AR signalling and the genes and signalling pathways that it controls must occur in tandem with this so the molecular events are fully characterised.
- These two research points will combine to produce tailored drug therapy for individual cases of infertility and may also contribute to the development of novel male contraceptives.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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References

[1] Russell LD, Clermont Y. Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. Anat Rec 1977;187(3):347–66.
[2] Bartlett JM, Kerr JB, Sharpe RM. The effect of selective destruction and regeneration of rat Leydig cells on the intratesticular distribution of testosterone and morphology of the seminiferous epithelium. J Androl 1986;7(4):240–53.
[3] Zhang FP, Poutanen M, Wilbertz J, et al. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. Mol Endocrinol 2001;15(1):172–83.
[4] Lubahn DB, Joseph DR, Sullivan PM, et al. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 1988;240(4850):327–30.
*[5] Bennett NC, Gardiner RA, Hooper JD, et al. Molecular cell biology of androgen receptor signalling. Int J Biochem Cell Biol 2010;42(6):813–27.
*[6] Walker WH. Non-classical actions of testosterone and spermatogenesis. Philos Trans R Soc Lond Ser B Biol Sci 2010; 365(1546):1557–69.

[7] Tsai MY, Yeh SD, Wang RS, et al. Differential effects of spermatogenesis and fertility in mice lacking androgen receptor in individual testis cells. Proc Natl Acad Sci U. S. A 2006;103(50):18975–80.
[8] Johnston DS, Russell LD, Friel PJ, et al. Murine germ cells do not require functional androgen receptors to complete spermatogenesis following spermatogonial stem cell transplantation. Endocrinology 2001;142(6):2405–8.
[9] Lyon MF, Glenister PH, Lamoreux ML. Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. Nature 1975;258(5536):620–2.
[10] Zhou Q, Nie R, Prins GS, et al. Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J Androl 2002;23(6):870–81.
[11] Lyon MF, Hawkes SG. X-linked gene for testicular feminization in the mouse. Nature 1970;227(5264):1217–9.
[12] Gaspar ML, Meo T, Bourgarel P, et al. A single base deletion in the Tfm androgen receptor gene creates a short-lived messenger RNA that directs internal translation initiation. Proc Natl Acad Sci U. S. A 1991;88(19):8606–10.
*[13] Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis 2000;26(2):99–109.
[14] De Gendt K, Swinnen JV, Saunders PT, et al. A sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci U. S. A 2004;101(5):1327–32.
[15] Notini AJ, Davey RA, McManus JF, et al. Genomic actions of the androgen receptor are required for normal male sexual differentiation in a mouse model. J Mol Endocrinol 2005;35(3):547–55.
[16] Sato T, Matsumoto T, Kawano H, et al. Brain masculinization requires androgen receptor function. Proc Natl Acad Sci U. S. A 2004;101(6):1673–8.
[17] Yeh S, Tsai MY, Xu Q, et al. Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci U. S. A 2002;99(21):13498–503.
[18] Hannema SE, Scott IS, Rajpert-De Meyts E, et al. Testicular development in the complete androgen insensitivity syndrome. J Pathology 2006;208(4):518–27.
*[19] Smith L. Good planning and serendipity: exploiting the Cre/Lox system in the testis. Reproduction 2011;141(2):151–61.
[20] Mruk DD, Cheng CY. Sertoli–Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr Rev 2004;25(5):747–806.
[21] Vogl AW, Pfeiffer DC, Redenbach DM, et al. Sertoli cell cytoskeleton. In: Russell LD, D GM, editors. The Sertoli cell. Cache River Press; 1993. p. 39–86.
[22] Lie PP, Mruk DD, Lee WM, et al. Cytoskeletal dynamics and spermatogenesis. Philos Trans R Soc Lond Ser B Biol Sci 2010; 365(1546):1581–92.
[23] Willems A, De Gendt K, Allemeersch J, et al. Early effects of Sertoli cell-selective androgen receptor ablation on testicular gene expression. Int J Androl 2009;33(3):507–17.
[24] Berensztein EB, Baquedano MS, Gonzalez CR, et al. Expression of aromatase, estrogen receptor alpha and beta, androgen receptor, and cytochrome P-450scc in the human early prepubertal testis. Pediatr Res 2006;60(6):740–4.
[25] Rey RA, Musse M, Venara M, et al. Ontogeny of the androgen receptor expression in the fetal and postnatal testis: its relevance on Sertoli cell maturation and the onset of adult spermatogenesis. Microsc Res Tech 2009;72(11):787–95.
[26] Chemes HE, Rey RA, Nistal M, et al. Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. J Clin Endocrinol Metab 2008;93(11): 4408–12.
[27] Suarez-Quian CA, Martinez-Garcia F, Nistal M, et al. Androgen receptor distribution in adult human testis. J Clin Endocrinol Metab 1999;84(1):350–8.
[28] Hazra R, Corcoran L, Robson M, et al. Temporal role of Sertoli cell androgen receptor expression in spermatogenic development. Mol Endocrinol 2013;27(1):12–24.
[29] Chang C, Chen YT, Yeh SD, et al. Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proc Natl Acad Sci U. S. A 2004;101(18):6876–81.
[30] Lim P, Robson M, Spaliviero J, et al. Sertoli cell androgen receptor DNA binding domain is essential for the completion of spermatogenesis. Endocrinology 2009;150(10):4755–65.
[31] Tan KA, De Gendt K, Atanassova N, et al. The role of androgens in sertoli cell proliferation and functional maturation: studies in mice with total or Sertoli cell-selective ablation of the androgen receptor. Endocrinology 2005;146(6):2674–83.
[32] O'Shaughnessy PJ, Monteiro A, Abel M. Testicular development in mice lacking receptors for follicle stimulating hormone and androgen. PLoS One 2012;7(4):e35136.
[33] De Gendt K, Atanassova N, Tan KA, et al. Development and function of the adult generation of Leydig cells in mice with Sertoli cell-selective or total ablation of the androgen receptor. Endocrinology 2005;146(9):4117–26.
*[34] Holdcraft RW, Braun RE. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development 2004;131(2):459–67.
[35] Farr CH, Ellis LC. In-vitro contractility of rat seminiferous tubules in response to prostaglandins, cyclic GMP, testosterone and 2,4′-dibromoacetophenone. J Reprod Fertil 1980;58(1):37–42.
[36] Skinner MK, Tung PS, Fritz IB. Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. J Cell Biol 1985;100(6):1941–7.
[37] Richardson LL, Kleinman HK, Dym M. Basement membrane gene expression by Sertoli and peritubular myoid cells in vitro in the rat. Biol Reproduction 1995;52(2):320–30.
[38] Drews U, Sulak O, Oppitz M. Immunohistochemical localisation of androgen receptor during sex-specific morphogenesis in the fetal mouse. Histochem Cell Biol 2001;116(5):427–39.
[39] You L, Sar M. Androgen receptor expression in the testes and epididymides of prenatal and postnatal Sprague–Dawley rats. Endocrine 1998;9(3):253–61.
[40] Majdic G, Millar MR, Saunders PT. Immunolocalisation of androgen receptor to interstitial cells in fetal rat testes and to mesenchymal and epithelial cells of associated ducts. J Endocrinol 1995;147(2):285–93.
[41] Welsh M, Saunders PT, Atanassova N, et al. Androgen action via testicular peritubular myoid cells is essential for male fertility. FASEB J 2009;23(12):4218–30.
*[42] De Gendt K, Verhoeven G. Tissue- and cell-specific functions of the androgen receptor revealed through conditional knockout models in mice. Mol Cell Endocrinol 2012;352(1–2):13–25.

[43] McLachlan RI, O'Donnell L, Meachem SJ, et al. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Hormone Res 2002;57:149–79.
[44] Mayerhofer A. Human testicular peritubular cells: more than meets the eye. Reproduction 2013;145(5):R107–16.
[45] Skinner MK, Fritz IB. Testicular peritubular cells secrete a protein under androgen control that modulates Sertoli cell functions. Proc Natl Acad Sci U. S. A 1985;82(1):114–8.
[46] Verhoeven G, Hoeben E, De Gendt K. Peritubular cell-Sertoli cell interactions: factors involved in PmodS activity. Andrologia 2000;32(1):42–5.
[47] Dym M, Fawcett DW. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. Biol Reprod 1970;3(3):308–26.
[48] Meng J, Holdcraft RW, Shima JE, et al. Androgens regulate the permeability of the blood-testis barrier. Proc Natl Acad Sci U. S. A 2005;102(46):16696–700.
[49] Wang RS, Yeh S, Chen LM, et al. Androgen receptor in sertoli cell is essential for germ cell nursery and junctional complex formation in mouse testes. Endocrinology 2006;147(12):5624–33.
[50] Willems A, Batlouni SR, Esnal A, et al. Selective ablation of the androgen receptor in mouse sertoli cells affects sertoli cell maturation, barrier formation and cytoskeletal development. PLoS One 2010;5(11):e14168.
[51] Denolet E, De Gendt K, Allemeersch J, et al. The effect of a sertoli cell-selective knockout of the androgen receptor on testicular gene expression in prepubertal mice. Mol Endocrinol 2006;20(2):321–34.
[52] Eacker SM, Shima JE, Connolly CM, et al. Transcriptional profiling of androgen receptor (AR) mutants suggests instructive and permissive roles of AR signaling in germ cell development. Mol Endocrinol 2007;21(4):895–907.
[53] Rato L, Socorro S, Cavaco JE, et al. Tubular fluid secretion in the seminiferous epithelium: ion transporters and aquaporins in Sertoli cells. J Membr Biol 2010;236(2):215–24.
[54] McCabe MJ, Allan CM, Foo CF, et al. Androgen initiates Sertoli cell tight junction formation in the hypogonadal (hpg) mouse. Biol Reproduction 2012;87(2):38.
*[55] O'Donnell L, McLachlan RI, Wreford NG, et al. Testosterone withdrawal promotes stage-specific detachment of round spermatids from the rat seminiferous epithelium. Biol Reprod 1996;55(4):895–901.
[56] O'Donnell L, Stanton PG, Bartles JR, et al. Sertoli cell ectoplasmic specializations in the seminiferous epithelium of the testosterone-suppressed adult rat. Biol Reprod 2000;63(1):99–108.
[57] Saito K, O'Donnell L, McLachlan RI, et al. Spermiation failure is a major contributor to early spermatogenic suppression caused by hormone withdrawal in adult rats. Endocrinology 2000;141(8):2779–85.
[58] Guttman JA, Takai Y, Vogl AW. Evidence that tubulobulbar complexes in the seminiferous epithelium are involved with internalization of adhesion junctions. Biol Reprod 2004;71(2):548–59.
[59] Beardsley A, O'Donnell L. Characterization of normal spermiation and spermiation failure induced by hormone suppression in adult rats. Biol Reprod 2003;68(4):1299–307.
[60] Beardsley A, Robertson DM, O'Donnell L. A complex containing alpha6beta1-integrin and phosphorylated focal adhesion kinase between sertoli cells and elongated spermatids during spermatid release from the seminiferous epithelium. J Endocrinol 2006;190(3):759–70.
[61] Yan HH, Cheng CY. Laminin alpha 3 forms a complex with beta3 and gamma3 chains that serves as the ligand for alpha 6beta1-integrin at the apical ectoplasmic specialization in adult rat testes. J Biol Chem 2006;281(25):17286–303.
[62] Zhang FP, Pakarainen T, Poutanen M, et al. The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. Proc Natl Acad Sci U. S. A 2003;100(23):13692–7.
*[63] Verhoeven G, Willems A, Denolet E, et al. Androgens and spermatogenesis: lessons from transgenic mouse models. Philos Trans R Soc Lond Ser B Biol Sci 2010;365(1546):1537–56.
[64] Cheng CY, Mruk DD. An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: a biochemical and molecular perspective. Crit Rev Biochem Mol Biol 2009;44(5):245–63.
*[65] Gottlieb B, Beitel LK, Nadarajah A, et al. The androgen receptor gene mutations database: 2012 update. Hum Mutat 2012;33(5):887–94.
*[66] Hiort O, Holterhus PM, Horter T, et al. Significance of mutations in the androgen receptor gene in males with idiopathic infertility. J Clin Endocrinol Metab 2000;85(8):2810–5.
[67] Ghadessy FJ, Lim J, Abdullah AA, et al. Oligospermic infertility associated with an androgen receptor mutation that disrupts interdomain and coactivator (TIF2) interactions. J Clin Investig 1999;103(11):1517–25.
[68] Gao T, Marcelli M, McPhaul MJ. Transcriptional activation and transient expression of the human androgen receptor. J Steroid Biochem Mol Biol 1996;59(1):9–20.
[69] Choong CS, Kemppainen JA, Zhou ZX, et al. Reduced androgen receptor gene expression with first exon CAG repeat expansion. Mol Endocrinol 1996;10(12):1527–35.
[70] Tut TG, Ghadessy FJ, Trifiro MA, et al. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 1997;82(11):3777–82.
[71] Jaaskelainen J. Molecular biology of androgen insensitivity. Mol Cell Endocrinol 2012;352(1–2):4–12.
[72] De Gendt K, Verhoeven G, Amieux PS, et al. Genome-wide identification of AR-regulated genes translated in Sertoli cells in vivo using the Ribo Tag approach. Mol Endocrinol 2014;28(4):575–91.
[73] Panneerdoss S, Chang YF, Buddavarapu KC, et al. Androgen-responsive microRNAs in mouse Sertoli cells. PLoS One 2012;7(7):e41146.
[74] Jorgensen N, Joensen UN, Jensen TK, et al. Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. BMJ Open 2012;2(4).
[75] Sharpe RM. Sperm counts and fertility in men: a rocky road ahead. Science & society series on sex and science. EMBO Rep 2012;13(5):398–403.
[76] O'Hara L, McInnes K, Simitsidellis I, et al. Autocrine androgen action is essential for Leydig cell maturation and function, and protects against late-onset Leydig cell apoptosis in both mice and men. FASEB J 2014;29(3):894–910.
[77] Welsh M, Sharpe RM, Moffat L, et al. Androgen action via testicular arteriole smooth muscle cells is important for Leydig cell function, vasomotion and testicular fluid dynamics. PLoS One 2010;5(10):e13632.

[78] O'Hara L, Smith LB. Androgen receptor signalling in vascular endothelial cells is dispensable for spermatogenesis and male fertility. BMC Res Notes 2012;5:16.
[79] Wu CT, Altuwaijri S, Ricke WA, et al. Increased prostate cell proliferation and loss of cell differentiation in mice lacking prostate epithelial androgen receptor. Proc Natl Acad Sci U. S. A 2007;104(31):12679–84.
[80] Simanainen U, McNamara K, Davey RA, et al. Severe subfertility in mice with AR inactivation in sex accessory organs, but not in testis. Endocrinology 2008;149(7):3330–8.
[81] Welsh M, Moffat L, McNeilly A, et al. Smooth muscle cell-specific knockout of androgen receptor: a new model for prostatic disease. Endocrinology 2011;152(9):3541–51.
[82] Welsh M, Moffat L, Jack L, et al. Deletion of androgen receptor in the smooth muscle of the seminal vesicles impairs secretory function and alters its responsiveness to exogenous testosterone and estradiol. Endocrinology 2010;151(7):3374–85.
[83] Raskin K, de Gendt K, Duittoz A, et al. Conditional inactivation of androgen receptor gene in the nervous system: effects on male behavioral and neuroendocrine responses. J Neurosci 2009;29(14):4461–70.
[84] Juntti SA, Tollkuhn J, Wu MV, et al. The androgen receptor governs the execution, but not programming, of male sexual and territorial behaviors. Neuron 2010;66(2):260–72.
[85] O'Hara L, Welsh M, Saunders PT, et al. Androgen receptor expression in the caput epididymal epithelium is essential for development of the initial segment and epididymal spermatozoa transit. Endocrinology 2011;152(2):718–29.
[86] Krutskikh A, De Gendt K, Sharp V, et al. Targeted inactivation of the androgen receptor gene in murine proximal epididymis causes epithelial hypotrophy and obstructive azoospermia. Endocrinology 2011;152(2):689–96.