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Inhibiting Vitamin A Metabolism As An Approach To Male Contraception

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Abstract

While oral contraceptives have been available to women since the 1960s, contraceptive options for men have remained limited. Spermatogenesis relies on the active metabolite of vitamin A, retinoic acid, to drive spermatogonial differentiation and to allow the production of normal numbers of sperm. Recent evidence describes how the enzymes which control vitamin A metabolism in the testis could be targeted to generate effective male contraceptives; however, the detailed mechanism(s) regarding how vitamin A regulates normal spermatogenesis are still unknown. The essential nature of vitamin A to male germ cell development and the prospects of targeting the proteins responsible for the generation, transport, and storage of retinoic acid as targets for male contraceptive development are discussed in this review.

Spermatogenesis and the search for a male contraceptive

Providing men and women with the ability to choose when they want to bring a child into the world is essential in today's society. To suit the extensive range of people's needs and wants, the continual development and refinement of contraceptive technologies for both men and women is required. The use and distribution of an oral contraceptive for women was first approved in 1960 [1]. However, the production of a similar product for men has yet to be successful. An effective male contraceptive should either block sperm production or interfere with the ability of sperm to reach or fertilize an oocyte. For male contraceptives to be designed and produced, an understanding of the normal progression of spermatogenesis and whether there are particular events that can be manipulated in a testis-specific manner to eliminate the production of sperm, is required. Sperm production in mammals takes place in the testis and is controlled by three fundamental processes: the renewal and mitotic production of spermatogonia; the recombination and segregation of homologous chromosomes into daughter haploid cells during meiosis; and the unique morphological and nuclear changes, collectively known as spermiogenesis, which transform spermatids to spermatozoa (Figure 1).

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Given that access to human testicular tissue for research purposes is extremely limited, our current understanding of mammalian spermatogenesis has been generated via studies of rodent and nonhuman primate testis tissue. Comparisons of the spermatogenic process between rodents and primates, including humans, have been recently reviewed in several publications [2–5] and even though there is a general conservation of cell type and germ cell differentiation and division steps, there are some salient differences between primates and rodents. The majority of published data associated with vitamin A function in the testis has been generated using rodent models. For example, vitamin A is known to be essential for spermatogonial differentiation in rodents; whether this is also the case in primates is unknown. Because very little published information regarding vitamin A activity in the human testis is available, this review discusses the spermatogenic process and the testicular activity of vitamin A in rodents, but will link this information back to humans and how manipulating vitamin A activity could lead to the development of a male contraceptive.

In rodents, the spermatogonial population consists of the undifferentiated Type A spermatogonia (A_{single} , A_{paired} , A_{aligned}), which divide to continually repopulate the testis with stem cells as well as provide progenitor cells, the differentiated spermatogonia (A_1 , A_2 , A_3 , A_4 , Intermediate and B), which are committed to undergoing meiosis [6]. Once spermatogonia enter the differentiation pathway, a process known as the A to A_1 transition, they begin a series of irreversible steps leading to the conversion of these cells to preleptotene spermatocytes and meiotic initiation. Therefore, the A to A_1 transition in rodents represents the commitment of spermatogonia to enter meiosis. Meiosis is unique to germ cells. It generates genetic diversity through homologous recombination and allows the proper chromosome segregation so that each daughter cell is haploid. In the mouse, meiotic prophase is lengthy (approximately 2 weeks) and the cells are divided into different populations based on their chromatin morphology (preleptotene, leptotene, zygotene, pachytene, and diplotene) [6,7]. The result of meiosis in the testis is the production of four round haploid spermatids from each diploid germ cell, which then undergo spermiogenesis to first form elongating spermatids and finally spermatozoa [8].

The complex series of events which must occur for spermatozoa to be produced cannot be completed without the supporting somatic environment [6] (Figure 1). The seminiferous epithelium of the testis is composed of germ cells and somatic Sertoli cells, the latter which support spermatogenesis through cell-to-cell contact with the germ cells and by maintaining the structural integrity of the epithelium. This epithelium is surrounded by the peritubular myoid cells (PTMs) to form the seminiferous tubules. These PTMs are contractile and aid in the movement of spermatozoa out of the testis [6]. Within the interstitial space between tubules are the Leydig cells, which are responsible for testosterone production. In addition, the interstitial space contains macrophages, blood, lymphatic vessels and nerves. The function of the seminiferous epithelium is directed by the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are required for normal spermatogenesis. While LH stimulates testosterone biosynthesis, FSH maintains the function of the Sertoli cells, which nurture and promote germ cell differentiation.

One approach to male contraceptive development is to suppress LH and FSH secretion from the pituitary by negative feedback using combinations of steroid hormones, usually an androgen combined with a progestin. This deprives the testes of the signals required for normal spermatogenesis, leading to profound suppression of sperm production in most men. Unfortunately, this approach has not resulted in uniform suppression of spermatogenesis in clinical studies [9]. Therefore, alternative approaches to male contraceptive development are needed.

Vitamin A is essential for male germ cell development, as shown from studies in vitamin A deficient (VAD) mice [10] (Figure 1). This review will highlight the ways in which the metabolism of vitamin A to its active metabolite, retinoic acid (RA), and the downstream signaling events could be manipulated to develop a male contraceptive and describe the current data which suggests that limiting the availability of RA specifically to the testis could effectively and reversibly inhibit spermatogenesis.

The metabolism and signaling of vitamin A in the testis

In mammals, vitamin A is usually transported via serum as retinol (ROL) bound to retinoid binding proteins, and conversion of this retinoid for either storage (retinyl esters) or use (RA) takes place in target tissues [11]. Retinol is taken up by cells via the membrane-bound receptor STRA6 and once inside, the conversion of ROL to RA is controlled by a two-step enzymatic process [12] (Figure 2a). An understanding of which retinoid metabolizing enzymes are expressed in the mammalian testis is still being shaped. There is no published information regarding the cell-specific expression of the retinoid metabolizing enzymes in the human testis. However, those enzymes with known testicular cellular expression patterns in the rodent testis are detailed (Figure 2b). In 2006, human tissue distribution and expression profiles were published for the majority of the alcohol and aldehyde dehydrogenases [13]. Two aldehyde dehydrogenases, *Aldh1a2* and *Aldh9a1*, appear to be testis-enriched, suggesting that these two enzymes could be interesting targets for male contraceptive development. *Aldh1a2* was expressed 8-fold higher in the testis than any other tissue in men [13]. This enzyme has been recently identified as a target of a group of potential male contraceptive compounds [14], and this research will be discussed in detail in a later section of this review. A survey of aldehyde dehydrogenases in different mouse tissues also found *Aldh1a2* transcript to be enriched in the testis [15]. Isoelectric focusing demonstrated that ALDH1A2 displays its highest enzymatic activity in the mouse testis [16]. *Aldh1a2* transcript localization in the mouse testis has been investigated in two separate publications, with mRNA present in spermatogonia and spermatocytes [17] and in pachytene spermatocytes and spermatids in the adult mouse testis, but only in Sertoli cells at 5 days post partum (dpp) in the second study [18]. These expression studies suggest that the inhibition of ALDH1A2 activity, as a form of contraception, could possibly target multiple germ cell types; however, it will be important to understand which cell types contain ALDH1A2 protein, both in rodents and humans, to determine how manipulating its activity could serve as an effective contraceptive. *Aldh9a1* transcript was enriched in human skeletal muscle with the human adult testis displaying the second highest level of expression [13]. No cell-specific mammalian testis expression pattern has been described for *Aldh9a1* so it is unclear as to which cells would be affected if the activity of this enzyme was inhibited. *Aldh1a3* has been detected in Leydig cells [18], and conflicting localization patterns have been published for *Aldh1a1*. *Aldh1a1* was published as Leydig cell-specific [19], with a recent study also detecting transcript in Sertoli cells [18]; however, a third study localized the mRNA to spermatocytes, with some signal also present in spermatogonia and spermatids [17]. In terms of the testis localization of other retinoid metabolizing enzymes, a variety of cell populations are positive. *Rdh1* and *Rdh2* are both present in rodent Sertoli cells, spermatogonia and primary spermatocytes [20], *Rdh9* has been detected in spermatogonia and Leydig cells [21], RDH11 localized only to pachytene spermatocytes in the rodent testis and specifically to the Golgi body [22], *Adh1* mRNA and protein was detected in rodent Sertoli and Leydig cells and late spermatids have been shown to contain *Adh7* mRNA and protein [23]. Taken together, these expression data show that both germ and somatic cells in the testis are likely to be able to metabolize retinol, but there are discrepancies associated with which cell types express particular enzymes. Further research is required to further define how each germ cell type metabolizes retinol and whether the data generated in

rodents can be applied to the human testis to determine whether these enzymes can be targets for male contraceptive design.

For RA to be active, a cell must also contain components of the RA signaling machinery (Figure 3). Within all mammalian cells, it is currently thought that RA signals through a heterodimer of a retinoic acid receptor (RAR) and a retinoid X receptor (RXR) [12]. RAR α has been localized to Sertoli cells in the adult mouse testis [18]; however, studies in the rat testis have detected *Rara* mRNA and protein in round and elongating spermatids in the adult and meiotic prophase germ cells and Sertoli cells in juvenile animals [24]. Male *Rara* knockout mice are sterile, and testis transplantation studies have shown that *Rara*-deficient germ cells rarely colonize a recipient testis; however, once colonized, spermatogenesis proceeds normally [25]. It is currently unknown why *Rara*-deficient germ cells do not efficiently colonize a recipient testis, but one hypothesis is that these germ cells, with an impaired ability to respond to RA, do not differentiate efficiently [25]. In the reciprocal experiment, wildtype germ cells transplanted into a *Rara*-deficient testis colonize and proliferate normally; however, their development is mostly halted during meiotic prophase [25]. A second transplantation study also identified improper cellular associations and abnormal sperm production when *Rara*-deficient germ cells were introduced into a germ cell-depleted wildtype testis [26]. Therefore, RAR α is a key signaling molecule during testis development and functions in Sertoli cells in rodents to regulate meiosis but may also be important for the proliferation and differentiation of spermatogonia. Because of its importance in rodent spermatogenesis, RAR α may be an attractive target for male contraceptive development. RAR α specific antagonists have the potential to block spermatogonial differentiation without impacting other retinoid mediated processes which rely on other RAR receptors.

Using immunohistochemistry and *LacZ* reporter gene studies in mice, RAR γ was found in A spermatogonia [18]; however, *Rarg*-deficient male mice have no reported fertility problems [27]. Further studies are required to determine how RA signaling is regulated by RAR γ in spermatogonia and which mechanisms are in place to compensate for the loss of this receptor. RXR β has been shown to be critical for normal spermatogenesis [28]. Both *Rxr* β transcript and protein are present in mouse Sertoli cells [18]. Deleting *Rxr* β results in failed spermatid release, accumulation of cholesterol esters and testis degeneration. These phenotypes are conserved when the gene is deleted only in Sertoli cells [28]. Interestingly, of the global *Rxr* β knockout animals, 50% die at birth but those that survive are normal apart from male sterility. The deletion of CCR4-NOT transcription complex, subunit 7 (*Cnot7*) also produces sterile male mice with a phenotype similar to that of the *Rxr* β knockout mouse except that these animals are born in normal Mendelian ratios [29]. CNOT7 is a coregulator of RXR β in Sertoli cells; therefore, mutations in this protein affect the ability of RXR β to function correctly [29]. Mice deficient in either RAR β or RXR γ display normal fertility with the deletion of *Rxra* resulting in embryonic lethality [30–32]. In addition, there are differing reports regarding the localization of RAR β , RXR α and RXR γ in mammalian testes. One study reported that each of these receptors is exclusively expressed in round spermatids of the adult mouse testis [18]; however, a second study in the rat describes staining in Sertoli cells and germ cells for RAR β , and staining in the majority of germ cell populations for both RXR α and RXR γ [33].

The generation of knockout mice has been extremely useful in describing functions for the retinoid metabolizing and signaling proteins and may provide insight into which of these proteins could be potential targets for male contraceptive development [22,27,30–32,34–39]. Figure 4 outlines the steps during spermatogenesis which are affected by the elimination of either a retinoid metabolizing enzyme or signaling protein and indicates whether they could be exploitable for contraceptive design. If a knockout mouse displays a phenotype with only

male fertility affected, such is the case for $RAR\alpha$, then this protein is a potential contraceptive target. If embryonic lethality is the result of a gene knockout, as is the case for $Rxra$, or if a knockout results in no fertility phenotype at all, e.g. mice deficient in either $RAR\beta$ or $RXR\gamma$, these proteins are unlikely targets for contraceptive design. However, this is not always the case. Global *Aldh1a2* null mice display perinatal lethality [34] and yet, compounds which inhibit this enzyme in humans appear to be excellent contraceptive candidates [14]. In addition, mouse knockout studies provide no information regarding whether the elimination of a retinoid metabolizing or signaling protein also generates the same response in humans. To determine whether the proteins responsible for vitamin A metabolism and signaling can be manipulated so that sperm production or function is blocked in men, an understanding of how vitamin A metabolism and signaling occurs in the human testis is required.

The regulation of spermatogonial differentiation by vitamin A

The gene expression events that drive the A to A₁ transition are still being elucidated; however, it is known that RA plays an essential role in rodents [10]. When adult male mice are made VAD, via maintaining them on a vitamin A-deficient diet for 2–3 months, all differentiated germ cells are lost from the seminiferous epithelium, and only type A spermatogonia and Sertoli cells remain [10,40] (Figure 1). This indicates that removing RA blocks the ability of undifferentiated spermatogonia to differentiate in the adult rodent testis. It is currently unknown whether spermatogonial differentiation is also blocked if primates are made VAD. Human cases of VAD have been reported, primarily in third world countries [41]; however, the effect that VAD has on their fertility has yet to be published. A VAD study in nonhuman primates is necessary to determine whether eliminating RA in the primate testis also results in a block during spermatogonial differentiation.

It is clear that RA plays a key role in the differentiation of spermatogonia; however, we are only just beginning to understand which genes respond to RA in order for the A to A₁ transition to be completed and whether the data generated in rodents translates to the human testis. The classic marker of RA activity in the testis, and at meiotic entry in the ovary, is *Stra8*. *Stra8*-deficient mice are infertile as germ cell differentiation in both males and females is blocked during meiosis, with preleptotene cells accumulating in both the testes and ovaries [42,43], and RA has been shown to be necessary for the induction of *Stra8* in both sexes [44]. Transcriptome analysis of embryonic ovaries and postnatal testes from mice revealed that *Stra8* is most highly expressed at embryonic day 14.5 and 10 dpp, respectively, the timepoints corresponding to meiotic initiation in females and males [45,46]. In addition, *Stra8* expression in the human fetal ovary and testis correlates well with the mouse microarray data, with a peak in expression detected during the onset of meiosis in the human fetal ovary and no expression detected in the fetal testis [47]. A *Stra8* expression pattern in the human postnatal testis has yet to be described; however, given that the fetal human and mouse data correlate well, our current hypothesis is that STRA8 will also be important for meiotic progression in men. *Stra8* is an important RA target gene in terms of regulating meiotic progression in rodents; yet there must be other RA-responsive targets which are critical for the A to A₁ spermatogonial transition. The function of STRA8 is unknown, making its function less attractive for contraceptive development. Other gene targets which control spermatogonial differentiation and are regulated by RA remain to be discovered and this area of research is currently the focus of studies in multiple laboratories. However, the fact that spermatogonial differentiation can be inhibited by removing vitamin A from the diet of a mouse and that spermatogenesis can be reinitiated with vitamin A replacement makes the proteins responsible for metabolizing vitamin A and the downstream targets of RA signaling interesting targets for male contraceptive design.

Inhibiting vitamin A function - an effective male contraceptive?

The optimal target for testicular contraception should be organ-specific or enriched, essential for germ cell production and accessible to inhibition by small molecules. Vitamin A is essential for the development and health of many different organ systems and it appears as though numerous vitamin A metabolizing enzymes have evolved so that there are some which are enriched in specific tissue types [13]. In the adult testis, *Aldh1a2* and *Aldh9a1* appear to be enriched, and a recent study highlighted ALDH1A2 as the potential target of compounds which are known to reversibly inhibit spermatogenesis [14]. The bisdichloroacetyldiamines (BDADs) are organic compounds that contain two amine groups linked by a hydrocarbon chain of varying lengths and inhibit the activity of the aldehyde dehydrogenases. The BDAD WIN 18,446 has recently been shown to inhibit the conversion of retinal to RA [14]. Interestingly, these compounds were found in the 1960s to safely and reversibly inhibit spermatogenesis in men. However, further research into the mechanism of action of these compounds was halted when administration of these compounds to men induced a disulfiram reaction when alcohol was also consumed. Disulfiram reactions are mediated when exogenous compounds inhibit the conversion of acetaldehyde to acetic acid, a necessary step in the metabolism of alcohol. Such reactions are characterized by severe flushing, sweating, nausea, vomiting and other symptoms. [48]. In retrospect, it appears that BDADs such as WIN 18,446 were the first example of the idea that suppression of testicular retinoic acid biosynthesis could function as a male contraceptive.

Notably, mice treated with the compound have testes which are morphologically similar to VAD testes [49]. In addition, daily treatment of rabbits with WIN 18,446 significantly reduced sperm counts, intratesticular RA and *Stra8* expression, with the reduction in intratesticular RA preceding the histological appearance of impairments in spermatogenesis. Hormonal assays determined that androgen levels were normal in WIN 18,446 treated animals, indicating that WIN 18,446 is specific to inhibiting the conversion of ROL to RA and thereby blocking spermatogonial differentiation. Clearly, WIN 18,446 is not a candidate contraceptive compound due to the side effects when taken with alcohol; however, ALDH1A2 appears to be an excellent candidate for the design of a male contraceptive to specifically inhibit vitamin A metabolism in the testis. With regards to ALDH9A1, it is currently only known to metabolize ethylene glycol ethers; therefore, further research is needed to determine the function of this aldehyde dehydrogenase in the testis and whether inhibition of its function could result in blocking normal sperm development.

Gene knockout studies in mice may have also highlighted RA signaling proteins which could be potential targets of male contraceptive design. As mentioned, the deletions of *Stra8*, *Rara*, *Rxrβ*, or *Cnot7* all result in male infertility [25,28,29,42], yet the animals appear to be otherwise normal. This suggests that small inhibitory molecules designed specifically for these RA signaling molecules or their regulatory proteins which act only in the testis could be potential male contraceptives. However, these are not simple compounds to design. RARα antagonists are currently available for research purposes; however, instead of being specific for RARα, they merely favor this receptor over the other two RARs. For an inhibitor of a receptor to be an effective contraceptive, it would need to inhibit only one receptor. In terms of STRA8, nothing is known about how it functions to drive meiotic progression; therefore a better understanding of its normal activity is essential before it can be determined whether inhibiting this protein would be an effective male contraceptive. An enhanced understanding of the testis-specific regulators of RA signaling will also aid in identifying other potential targets for male contraceptive development.

Conclusions

Women currently have numerous choices for methods of contraception whereas men only have two, condoms or vasectomy, with the latter not an appropriate option for men who wish to conceive children in the future. There has been a great deal of work performed attempting to develop a hormonal male contraceptive [9]; however, issues regarding drug delivery, efficacy and side effects of hormone administration to men make this approach to male contraceptive development problematic. Indeed, despite over 30 years of research into male hormonal contraception, no product based on this approach has been introduced into clinical practice. Other approaches to male contraceptive development are very early in development and are unlikely to reach the marketplace anytime soon. For example, indenopyridines have been shown to have antispermatogenic activity in dogs and monkeys but their mechanism of action in primates is still unclear [9]. Treatment with adjuvin, a compound designed to target the junctions which bind Sertoli cells to spermatids, does result in reversible infertility but serious side effects have been observed during testing [9]. In addition, recent advances in genomics and proteomics have allowed the identification of epididymal- and sperm-specific proteins, but inhibitors for these proteins still need to be identified and screened. In contrast, given the demonstrated ability of compounds like WIN 18,446 to safely and reversibly inhibit spermatogenesis in man via inhibition of testicular retinoic acid biosynthesis, a retinoid-based contraceptive may be possible in the next several years (Box 1). Work is ongoing to develop novel inhibitors of retinoic acid biosynthesis which do not cause the disulfiram reaction or interfere with retinoic synthesis and function in non-testicular tissue. If successful, such a compound may finally bring the dream of a reversible male contraceptive to fruition.

Text Box 1: Inhibiting vitamin A metabolism as a male contraceptive – the unanswered questions

- Improved understanding of the effects of retinoic acid on the germ cells. How does RA initiate differentiation?
- Better characterization of the biosynthesis and metabolism of retinoic acid in the testes. How is intratesticular RA regulated?
- Development of novel testes-specific drugs to target RA biosynthesis. Are testes-specific inhibitors possible?
- Ensure safety, efficacy and reversibility of RA biosynthesis inhibitors as male contraceptives. Is a safe, effective male contraceptive possible?

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Figure 1.

Vitamin A deficiency results in a block during spermatogonial differentiation. Histological cross-section of (a) a normal mouse testis tubule and (b) vitamin A-deficient (VAD) tubule, stained with Harris Haematoxylin to visualize chromatin. (c) The arrangement of germ and Sertoli cells within the normal seminiferous epithelium is shown. Sertoli cells maintain contact with each germ cell type throughout their development. As spermatogenesis proceeds, the more differentiated germ cells move towards the lumen of the tubule so that spermatozoa can be released. The peritubular myoid cells form a base for the seminiferous epithelium and also aid in moving spermatozoa through the testis tubules to be released into the male reproductive tract. (d) In the VAD mouse model, A to A₁ transition in spermatogonia is eventually blocked and all differentiated germ cells are lost from the testis.

The result is seminiferous tubules which contain only undifferentiated spermatogonia and Sertoli cells. Scale bars represent 50 μm .

Figure 2.

The vitamin A metabolizing enzymes are expressed in all cells of the seminiferous epithelium. (a) Schematics detailing the conversion of retinol (ROL) to retinoic acid (RA) and the families of enzymes which drive each step in all mammals. The oxidation of ROL to retinaldehyde is a reversible step and is controlled by the alcohol/retinol dehydrogenases and by the short-chain dehydrogenases. The conversion of retinaldehyde to RA is an irreversible step regulated by the aldehyde dehydrogenases. (b) Rodent adult testis cell-specific expression of the alcohol/retinol dehydrogenases and aldehyde dehydrogenases. Expression of each transcript and/or protein in a given cell type is marked by a colored bar above (alcohol/retinol dehydrogenases) or below (aldehyde dehydrogenases) that cell. The reference number is given directly under the colored bar for expression patterns of particular transcripts or proteins which were found to be different in the literature. Different colors represent each transcript and/or protein.

Figure 3.

Expression of the RARs and RXRs in the adult rodent testis. (a) Schematics detailing how gene expression is regulated by RA and its receptors. In the absence of RA, heterodimers of an RAR (red) and an RXR (purple) bind to RA response element (RARE) sequences (yellow) upstream of RA responsive genes (pink) and recruit histone deacetylases (HDAC, blue) to the site which condense chromatin and silence transcription of that particular gene. When RA (red triangle) is present, histone acetylases (HAT, green) are recruited, chromatin is decondensed and transcription proceeds. (b) Expression of the RARs and RXRs in cells of the adult rodent testis. Expression of each transcript and/or protein in a given cell type is marked by a colored bar above (RARs) or below (RXRs) that cell. The reference number is given directly under the colored bar for expression patterns of particular transcripts or proteins which were found to be different in the literature. *, Leydig cell expression of *Rxrα* [18] and localization of RARγ [33] was detected in juvenile rodent testes; however, the signal for these transcripts and/or proteins was below the level of detection for the systems used in studies in the adult rodent testis.

Embryonic lethal/die prenatally: <ul style="list-style-type: none"> •<i>Aldh1a2</i> [34] •<i>Aldh1a3</i> [35] •<i>Rxra</i> [30] 	Viable and fertile: <ul style="list-style-type: none"> •<i>Rxry</i> [32] •<i>Rarβ</i> [31] •<i>Rary</i> [27] •<i>Rdh1</i> [37] •<i>Rdh11</i> [22] •<i>Adh1</i> [38] •<i>Adh7</i> [39] •<i>Aldh1a1</i> [36]
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Knockouts with testis-specific phenotypes

Rara:

- Blocked at spermatogonial differentiation, looks similar to VAD [25]
- Some postnatal lethality, but those which live are normal except for male infertility
- Excellent contraceptive target

Stra8:

- Blocked at preleptotene spermatocytes [36]
- Normal except for male infertility
- Unknown function therefore difficult to target for contraceptive development

Rxrβ:

- Failed spermatid release, may also affect Sertoli cells [28]
- Some postnatal lethality, but those which live are normal except for male infertility
- Excellent contraceptive target

Cnot7:

- Failed spermatid release, may also affect Sertoli cells [29]
- Normal except for male infertility
- Excellent contraceptive target

Steps exploitable for male contraceptive development

Undifferentiated spermatogonia

Mitosis

Differentiated spermatogonia

Meiosis

Spermatocytes

Spermiogenesis

Spermatids

Spermatozoa

Inhibiting spermatogonial differentiation:

- Regulated by RA therefore could be targeted by inhibitors of RA metabolism or signaling.
- These cells lie outside the blood-testis barrier and therefore, are easily accessible.
- Potentially reversible.
- May not be as many mitotic proteins which are testis specific, reduced targets.
- May result in a ~25% reduction in testicular volume.

Inhibiting meiosis:

- Meiosis is a germ cell-specific event therefore, there are multiple meiosis-specific targets, e.g. *Stra8*, which could be potential contraceptive targets.
- May result in a ~25% reduction in testicular volume

Inhibiting spermiogenesis and spermatid release:

- Genomic and proteomic profiling has shown that spermatids contain numerous testis-specific proteins and therefore, large numbers of potential targets.
- Blocking spermatid release unlikely to lead to reduced testis size as the majority of germ cells remain inside the testis.
- Potentially reversible.
- Spermatids lie inside the blood-testis barrier and therefore, are not readily accessible.

Figure 4.

The generation of transgenic mouselines with knockouts of the retinoid metabolizing and signaling proteins has revealed interesting targets for the development of male contraceptives. The phenotypes of these knockouts are discussed throughout this manuscript, detailed and associated with the particular step of spermatogenesis which is inhibited. The reference number for each mouseline is noted. No data are presented for either *Aldh9a1* or *Rdh2* as no knockout phenotype has been published for either of these genes. The steps exploitable for male contraceptive design associated with inhibiting vitamin A metabolism or signaling are described in text boxes directly beside the cell type/s associated with that step.