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Review

Checking the Pulse of Vitamin A Metabolism and Signaling during Mammalian Spermatogenesis

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Abstract: Vitamin A has been shown to be essential for a multitude of biological processes vital for mammalian development and homeostasis. Its active metabolite, retinoic acid (RA), is important for establishing and maintaining proper germ cell development. During spermatogenesis, the germ cells orient themselves in very distinct patterns, which have been organized into stages. There is evidence to show that, in the mouse, RA is needed for many steps during germ cell development. Interestingly, RA has been implicated as playing a role within the same two Stages: VII and VIII, where meiosis is initiated and spermiation occurs. The goal of this review is to outline this evidence, exploring the relevant players in retinoid metabolism, storage, transport, and signaling. Finally, this review will provide a potential model for how RA activity is organized across the murine stages of the spermatogenic cycle.

Keywords: retinoic acid; Vitamin A; testis; spermatogenesis; pulse

1. Introduction

Retinoic acid (RA) plays a vital role in many different developmental processes in mammals. Embryonically, RA is important in organogenesis, limb-bud development, proper neuronal development, and germ cell fate in the developing gonad. In the urogenital ridge, RA signaling within the embryonic

ovary is responsible for fetal entry of oogonia into meiosis while RA degradation in the embryonic testis opposes this action [1,2], although there has recently been some debate on this front [3,4]. Postnatally, RA is an important molecule for the proper function of many organs, such as skin, lung, kidney, liver, and testis function.

Both high and low levels of RA have been shown to cause aberrant male germ cell development. Investigating the role that RA plays during spermatogenesis is vital to gain a better understanding of this complex biological process, but there are also practical applications for furthering this research. Currently, fifteen percent of couples in the United States suffer from infertility [5]. In approximately half of these diagnoses, the cause can be attributed to the male partner [6]. Unfortunately, the root of male infertility, in most cases, is unknown. Abnormal levels of RA have been associated with sterility [7], therefore, understanding the mechanism of control of RA within the testes will provide a better understanding of the players involved in spermatogenesis and has the potential to provide therapeutic options for those suffering from infertility. In addition, men are seeking to take a more active role in their reproductive health. Unfortunately, there is an appalling gap in healthcare equality when it comes to contraception. Women have a variety of contraceptive options, while the only impermanent option available to men is condoms. Because vitamin A metabolism plays such a crucial role in spermatogenesis, perturbing RA synthesis and signaling is an excellent target for non-hormonal, male contraception.

In the following sections, this review will provide a brief overview of both spermatogenesis and vitamin A metabolism, and will summarize the current evidence supporting the hypothesis that RA is the master regulator of the cycle of the seminiferous epithelium.

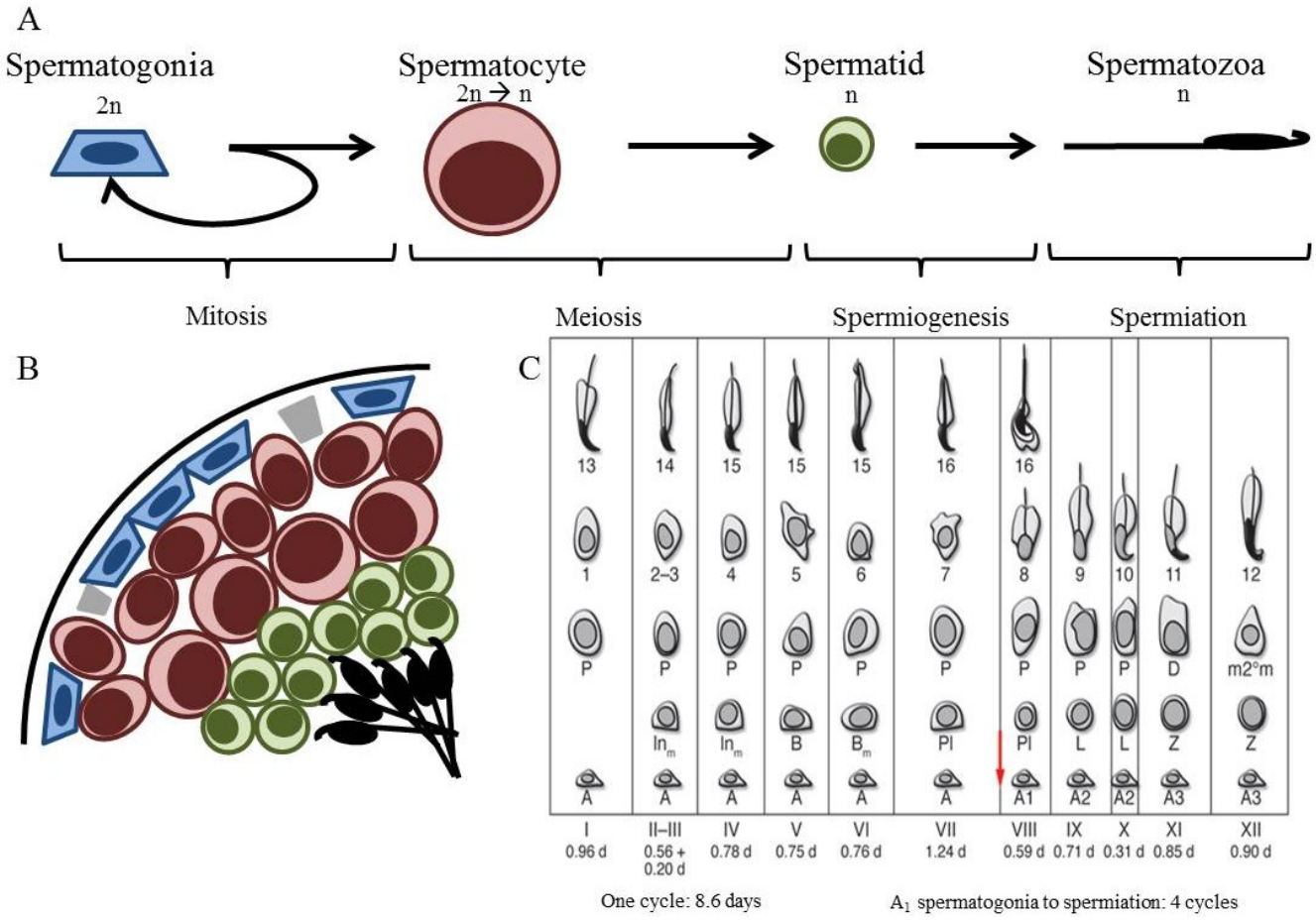
2. Spermatogenesis

Spermatogenesis is a tightly controlled process that can be divided into three distinct phases: (1) mitosis of spermatogonia, (2) meiotic division of spermatocytes, and (3) the morphological transformation of haploid spermatids to mature spermatozoa (Figure 1A). Diploid spermatogonia undergo several rounds of mitosis to both maintain a healthy stem cell pool and amplify the population of cells committed to undergoing meiosis. The spermatogonial population consists of both undifferentiated and differentiating spermatogonia, and the commitment to differentiate is known to be under the control of RA. Spermatocytes undergo meiosis for the purpose of halving their chromosomal number and ensuring genetic diversity. The first meiotic prophase is elongated during mammalian spermatogenesis when compared to the second, taking approximately twelve days in the mouse. RA is hypothesized to play a role during the initiation of this process. Finally, haploid spermatids undergo gross morphological changes, deemed spermiogenesis, before they are released as mature spermatozoa into the lumen of the seminiferous epithelium. Evidence suggests that both the radical morphological changes, as well as the release of the spermatozoa into the tubule lumen are under control of RA signaling. These spermatozoa are then shuttled to the rete testes where they are released into the epididymis for further maturation.

As germ cells progress through spermatogenesis, they travel from the basement membrane of the seminiferous tubule, where they start as undifferentiated spermatogonia, to the lumen, where they are released as spermatozoa. The columnar Sertoli cells are physically intertwined with the developing

germ cells and provide support, nutrients, and protection (Figure 1B). Much of our current understanding of mammalian spermatogenesis has been derived from studies in rodents and this review will primarily focus on data generated using mouse models. In the mouse, it takes approximately 35 days for an undifferentiated spermatogonium to differentiate, progress through meiosis, undergo spermiogenesis, and be released into the lumen of the tubule [8].

Figure 1. Overview of Murine Spermatogenesis. (**A**) Schematic representing the types of germ cells present within the seminiferous tubule. (**B**) Schematic representing the organization of the adult seminiferous tubule. Cell types in B correspond with those in A. Gray blocks represent Sertoli cell nuclei, while the white spaces between cells represent Sertoli cell cytoplasm. (**C**) Diagram depicting the twelve cellular associations present in murine spermatogenesis. The red arrow represents spermatogonial differentiation. (Figure 1C was adapted from [9]).



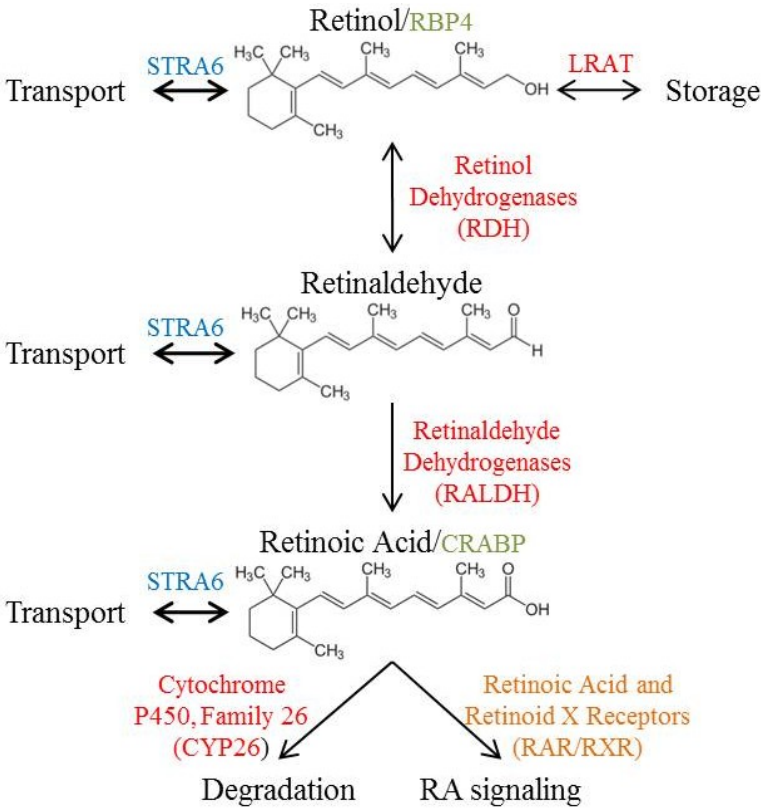
Spermatogenesis takes place in a very organized manner within the seminiferous tubule. In a normal adult murine testis, specific cell types are always observed together and these associations have been classified into stages [8] (Figure 1C). For example, upon examining a cross section of a seminiferous tubule, one will always observe spermatozoa being released into the lumen when round spermatids, preleptotene spermatocytes, and differentiating spermatogonia are present. Every tubule that contains these properties is a Stage VIII tubule in the mouse testis. If we were to look at a single cross section of a seminiferous tubule over time, we would see this tubule transition through all twelve spermatogenic

stages over the course of 8.6 days [8], known as the spermatogenic cycle. In order to continuously produce sperm, spermatogenesis occurs in an asynchronous manner; *i.e.*, all stages of the spermatogenic cycle are represented throughout the testis at any given moment. Current evidence indicates that RA function is essential in two of the twelve stages of murine spermatogenesis: Stages VII and VIII.

3. Vitamin A Metabolism

RA is vital for many different steps during spermatogenesis but cannot be readily transported throughout the body. Retinol (ROL), however, can be ingested through the diet and shuttled easily through the blood stream in complex with retinoid binding proteins (RBPs). When ROL reaches its target tissue, it is transported into the cell via the transmembrane receptor, STRA6, in tandem with the retinol binding protein RBP4, and can either be stored as retinyl esters by Lecitin retinol transferase (LRAT) or metabolized [10] (Figure 2). The metabolism of ROL to RA is a two-step enzymatic reaction, the first of which is the synthesis of retinaldehyde (RAL) via retinol dehydrogenase (RDH) enzymes. This reaction is thought to be the rate limiting step of vitamin A metabolism and is reversible [10]. The second is the conversion of RAL to RA, catalyzed by retinaldehyde dehydrogenase (RALDH) enzymes.

Figure 2. Vitamin A metabolism. This diagram represents vitamin A metabolism as it relates to spermatogenesis. Retinol (ROL), which is transported in complex with RBP4, is either stored or metabolized to RA via a two-step enzymatic process. Retinoic acid (RA) is then either degraded or utilized to allow transcription factors, in complex with cellular retinoic acid binding protein (CRABP), to stimulate gene expression. Relevant enzymes are labeled in red. Transcription factors are labeled in orange. Retinoid binding proteins are labeled in green. Membrane bound transporters are labeled in blue.



Once RA is synthesized, it can either be degraded by the cytochrome P450, family 26 (CYP26) enzymes into inert metabolites, or it can bind to the retinoic acid receptors (RARs), which then dimerize with retinoid X receptors (RXRs) [10]. RAR/RXR dimers bind to retinoic acid response elements (RAREs) located throughout the genome and induce differential expression of specific genes [11]. Current data indicate that RA signaling occurs in a stage-specific manner during the spermatogenic cycle.

4. The Role of RA during Spermatogenesis

4.1. Spermatogonial Differentiation

The first step of spermatogenesis is the differentiation of spermatogonia. In the mouse testis, the undifferentiated A spermatogonial population continuously divide in order to repopulate the testis, but a subset of these cells differentiate into A1 spermatogonia, known as the A to A1 transition, every 8.6 days. The A1 spermatogonia express KIT, the most well-known marker of differentiating spermatogonia, along with STRA8. These A1 spermatogonia undergo six mitotic divisions before entering meiosis [12]. In the adult mouse testis, the A to A1 transition takes place during Stage VIII.

Vitamin A is essential for spermatogonial differentiation. When male rodents are made vitamin A deficient (VAD), spermatogenesis is halted [13,14]. The only cell types present in the seminiferous epithelium of these animals are undifferentiated, KIT-negative spermatogonia and Sertoli cells [13–15]. When vitamin A, in the form of ROL or RA, is reintroduced, the spermatogonia differentiate simultaneously, becoming both KIT- and STRA8-positive [13,14]. There is sufficient evidence to indicate that RA is directly responsible for driving the expression of *Stra8* within the testis [16,17] and a growing body of data to suggest that it also regulates the transcription and translation of Kit [16,18,19]. Furthermore, spermatogenesis reinitiates normally, albeit in a synchronous manner [14,20], *i.e.*, only a few stages of the cycle are present and spermiation occurs throughout the entire testis simultaneously. Transgenic models have also been utilized to investigate the A to A1 transition in the absence of vitamin A in the juvenile testis. Both *Lrat*- and *Rbp4*-null mice, while perfectly fertile on a normal diet, become VAD much quicker than their wild type counterparts, likely due to depleted retinoid stores and aberrant transport capabilities [21,22]. The juvenile testes of both these VAD transgenic animals display a halt in spermatogenesis at spermatogonial differentiation [21,22]. Taken together, these data support the idea that vitamin A, or a downstream metabolite, is responsible for the A to A1 transition.

Results similar to what was observed in VAD testes were also seen when RALDH was inhibited either chemically or genetically. When animals were treated with an RALDH inhibitor, WIN 18,446, their testes were morphologically similar to that of a VAD animal, *i.e.*, the only cell types present within the tubules were undifferentiated spermatogonia and Sertoli cells [23,24]. When animals treated with WIN 18,446 were subsequently dosed with exogenous RA, spermatogenesis resumed normally but, again, in a synchronous manner [23]. A separate study examined testes from mice with Sertoli cell- specific deletions of *Aldh1a1*, *Aldh1a2*, and *Aldh1a3* (*Raldh1-3*), thereby eliminating RALDH activity in these cells [25]. The testes of these mice also exhibited a VAD-like phenotype, and when these animals were exposed to exogenous RA, spermatogenesis resumed, not only through the first

wave, but also through subsequent waves. [25]. All of these RA-depleted systems provide strong evidence indicating a role for RA during spermatogonial differentiation. The *Raldh1-3* Sertoli cell-specific deletion study demonstrates that the A to A1 transition requires RA to be synthesized by Sertoli cells, but only for the first spermatogenic wave.

Ablation of *Rdh10* in Sertoli cells of mice also causes a halt in spermatogenesis at the A to A1 transition, although the phenotype is more pronounced when *Rdh10* is removed from both Sertoli and germ cells [26]. Consistent with previous reports, the phenotype is rescued in a synchronous manner when retinoids were administered exogenously [26]. Interestingly, spermatogenesis is restored to normal in the absence of retinoid treatment after approximately 3 weeks in the *Rdh10*-deficient mice [26], indicating that RDH10 is required for the first and possibly second wave of spermatogenesis but is dispensable for subsequent waves. Because RA plays a role in the maintenance of spermatogenesis, it is probable that expression of a separate RDH enzyme is responsible for recovered fertility.

Furthermore, aberrant RA signaling inhibits spermatogonial differentiation. When RARγ is deleted either globally or specifically in spermatogonia, a VAD-like phenotype is observed in mice aged 10 weeks or older [27]. Ablation of both RARγ and RARα exacerbates this phenotype [27]. This indicates that RA signaling within the spermatogonial population is absolutely essential for differentiation to take place.

Finally, exogenous RA has been used to better understand the regulation of the A to A1 transition. Injection of exogenous RA into a neonatal mouse younger than 6 days postpartum (dpp) is sufficient to stimulate simultaneous spermatogonial differentiation, resulting in a synchronized testis [28,29]. Interestingly, it becomes impossible to synchronize spermatogenesis in this manner in mice after 8 dpp. What is special about an 8 dpp testis? In the mouse, this corresponds with the first appearance of preleptotene spermatocytes. It is possible that, after the first wave of spermatogenesis, the preleptotenes are the source of RA for the undifferentiated spermatogonia, and retinoid metabolism in the Sertoli cells is no longer required for differentiation. This theory is supported by the observation that spermatogonia in the testes of *Raldh1-3* Sertoli cell-specific knockout mice are capable of differentiating continuously following a single dose of exogenous RA [25].

4.2. Meiosis

Meiosis is the process by which diploid germ cells undergo one round of chromosomal duplication and two rounds of cell division, resulting in four genetically dissimilar haploid daughter cells. Meiosis takes approximately a cycle and a half of the seminiferous epithelium to fully complete in both mice and rats. In adult mice, meiotic initiation takes place during Stage VII.

Vitamin A plays a key role during male meiosis, with most of the current evidence derived from studies involving the expression and function of *Stra8*. This gene has been shown to be stimulated in the presence of RA—in fact *Stra8* stands for "stimulated by retinoic acid, gene 8"—and has been considered, for many years, to be the classical RA responsive gene [17,30]. Additionally, inhibition of RADLH activity in mice, via treatment with WIN 18,446 both *in vitro* and *in vivo*, was shown to suppress expression of *Stra8* [23,31]. *Stra8*-deficient germ cells fail to properly undergo meiosis in mice [32,33], indicating that *Stra8* is vital for normal meiotic progression.

Additional evidence to support a role for RA in the regulation of meiosis has been generated using the embryonic murine gonad as a model. Exogenous RA stimulated the expression of well characterized markers of meiosis, *Scp3*, *Dmc1*, and *γH2afx*, in the embryonic testis [34]. Moreover, inhibition of CYP26 enzymes with ketoconazole in the fetal testis resulted in an up-regulation of *Stra8*, *Scp3*, and *Dmc1*, and the germ cells took on morphological characteristics consistent with meiotic germ cells [1,34]. When fetal testes were treated with both ketoconazole and a pan-RAR antagonist, an induction of meiosis was not observed [1]. In a similar study, larval testes from several species of frogs were treated with exogenous RA or CYP26 inhibitors, and in both cases, leptotene spermatocytes were observed in the treated testes but not in the vehicle controls [35]. Moreover, when larval frog testes were cultured with either an RALDH inhibitor or an RAR antagonist, formation of leptotene spermatocytes was not observed [35]. Both of these studies implicate RA—specifically RA signaling—as being vital for meiotic progression.

Complementary evidence implicating RA as being responsible for meiotic initiation has also stemmed from studies of mice deficient in CYP26B1. A three-fold increase in RA-induced stimulation of a reporter construct was noted in the embryonic testis of *Cyp26b1*-null mice, indicative of higher RA levels [2]. An increase in *Stra8* and *Scp3* expression was also observed as well as the presence of cells morphologically similar to pachytene spermatocytes by embryonic day 16.5 [2,34]. Taken together, these data indicate that both RA accumulation and signaling are sufficient to drive meiosis.

4.3. Blood-Testes Barrier (BTB)

Vitamin A has been implicated in playing a vital role in the maintenance and reorganization of the blood-testis barrier (BTB), which is established by tight-junctions between adjacent Sertoli cells, forming two compartments for the germ cells to reside within: basal and adluminal. One purpose of the BTB is to provide an immune privileged environment for the developing germ cells. During and after meiosis, the germ cells produce antigenic proteins that are not produced anywhere else in the body and are, therefore, vulnerable to attack and clearance by the immune system. Diploid spermatogonia reside in the basal compartment while spermatocytes and spermatids reside in the adluminal compartment. As differentiating spermatogonia enter into meiosis, they must pass through the BTB in order to reside in an immune privileged environment. During Stage VIII, new tight-junctions are formed on the basal side of the preleptotene spermatocyte as the old tight-junctions on the adluminal side of the cell are degraded [36].

The integrity of the BTB is vital for fertility. When *Ocln*, a gene known to be essential for the BTB, was deleted in mice, massive germ cell loss was observed in the resulting animals [37]. Since then, it has been shown that RA induces expression of *Ocln* and *Zo1*, BTB genes encoding proteins that play crucial roles in tight-junctions, *in vitro* and *in vivo* respectively [38,39]. Tight-junction formation was stimulated by RA in cultured primary Sertoli cells isolated from a 20 dpp mouse [40]. In VAD mice, misregulation of genes known to be integral to the BTB, such as *Ocln*, *Cldn11*, and *Tjp1*, were observed via quantitative PCR [41]. In these same animals, OCLN and CLDN11 protein were abnormally localized in the absence of vitamin A [41]. Together, these data suggest that vitamin A is responsible for the appropriate expression and localization of vital BTB proteins.

Investigation of *Rara*-null mice has provided further evidence that RA is important for BTB integrity. Testes from these animals exhibited aberrant cell-cell interactions as well as a delay in incorporation of ZO1 [42]. A separate study investigated BTB function in adult mice that over-expressed a dominant-negative form of RARα specifically in Sertoli cells [38]. Five days after lentiviral injection, which was used to deliver the transgene, spermatocytes and spermatids underwent apoptosis, but only in Stages VII-XII [38]. Furthermore, ZO1 was down-regulated in Stages VII-XII, and a biotin permeability assay, which is used to assess the integrity of the BTB, showed that the barrier was compromised in these same stages [38]. Finally, the authors noted that the Sertoli cells appeared to have detached from the basement membrane of the seminiferous tubule in Stages VII-XII [38]. Interestingly, the investigators in this study examined the testis five days after lentiviral injection and observed all problems taking place in Stages VII-XII. It takes approximately 4.6 days for a Stage VII tubule to progress to a Stage XII tubule [8], indicating that RA signaling within the Sertoli cell only occurs during Stage VII. In total, these data provide strong evidence that RA signaling via the Sertoli cells at Stage VII of the cycle of the seminiferous epithelium is important for maintaining BTB integrity.

4.4. Spermiogenesis and Spermiation

Spermiogenesis occurs following meiosis when the newly formed round spermatids undergo gross morphological changes to become elongated spermatozoa. These spermatozoa are then released into the lumen of the seminiferous tubule during Stage VIII of the cycle, in a process called spermiation. Perturbations in RA metabolism and signaling have shown defects in both of these processes, but the extent to which RA plays a role has not been sufficiently examined.

Spermiation occurs abnormally in VAD rodents. In both *Rbp4*- and *Lrat*-null mice fed a VAD diet, spermatids were retained well past Stage VIII [21,43], and in VAD rats, spermatozoa were retained in many tubules as late as Stage XI [44]. In addition, when RA synthesis was ablated in Sertoli cells of mice, spermatids failed to properly align and were retained within the seminiferous epithelium later than normal [25]. These data show that the presence of RA, particularly in Sertoli cells, is essential for proper release of spermatozoa into the lumen of the tubule.

There is also evidence to indicate that RAR signaling is required for proper spermiogenesis and spermiation. When a dominant-negative form of RARα was over-expressed in Sertoli cells of adult mice, spermatids failed to release during Stage VIII and remained present at Stage IX [38], similar to what was observed in the RA deficient rats and mice. When *Rara* was globally excised in mice, the resulting testes showed a developmental arrest at step 8–9 spermatids during the first wave of spermatogenesis as well as a failure of these spermatids to properly align for release one cycle later during Stage VIII [45], but fertility was restored in these animals when *Rara* was overexpressed in haploid spermatids [46]. A separate study from the same laboratory demonstrated that RARα-deficient stem cells were capable of repopulating a germ cell-depleted wild type testis but produced spermatozoa exhibiting abnormal morphology, such as blunted heads [46]. Additionally, the researchers noted atypical chromatin condensation, reduced total cell number, and irregular cellular associations in the spermatids of these animals [46]. When RAR activity is chemically inhibited via a pan-RAR antagonist (BMS-189453), defects in spermiogenesis and spermiation also occur. Spermatids failed to properly align and release during Stages VIII and IX [47], and one month after a week of treatment with BMS-189453, some

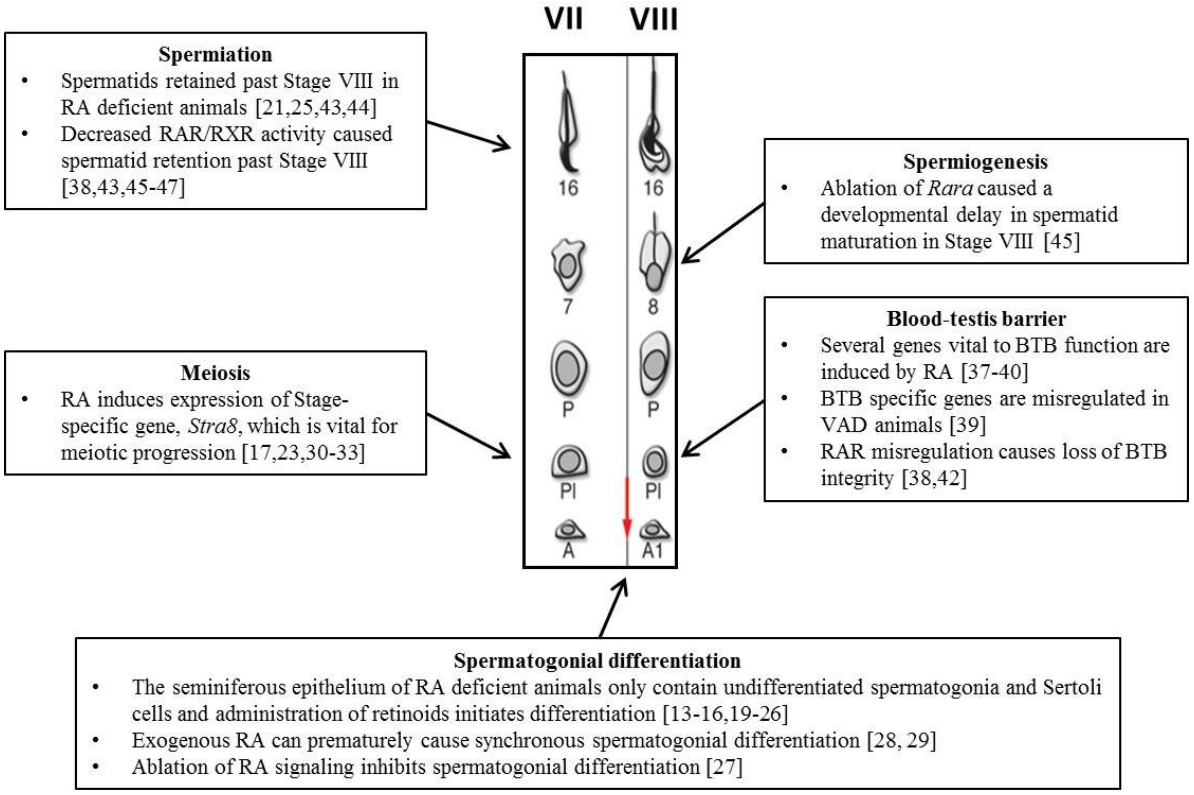
tubules were missing entire layers of germ cells [47]. All of these data strongly suggest that retinoid signaling via RARα is playing a crucial role in both spermatid maturation and release into the lumen of the seminiferous tubule.

Finally, defects in spermiation were observed in the *Rxrb*-deficient mouse. When this transcription factor was ablated specifically in Sertoli cells, spermatids failed to align on the luminal side of the seminiferous tubule and were not released through Stages IX and X [43]. Significantly more TUNEL-positive, apoptotic cells were also observed in the testes of these animals, which the authors hypothesized as being the result of phagocytized spermatids that were unable to be released into the tubule lumen [43]. These data indicate that signaling through RXRβ in Sertoli cells is vital to proper spermiation. As both RARα and RXRβ have been localized to Sertoli cells [48], it is likely that these two transcription factors heterodimerize to facilitate spermiation during Stage VIII.

5. What is the Cause of Stage-Specific RA Response?

As detailed above, RA is vital for many different steps in spermatogenesis and, interestingly, all of these processes occur during the same spermatogenic stages (Figure 3). Spermatogonial differentiation, meiotic initiation, reorganization of the BTB, and spermiation all occur during Stages VII and VIII of the spermatogenic cycle.

Figure 3. Stage-specificity of RA activity. This figure briefly outlines the relevant data that provide evidence supporting Stage-specificity of RA activity during Stages VII and VIII. RA has been shown to be vital during spermatogonial differentiation, meiotic initiation, blood-testis barrier (BTB) reorganization, spermiogenesis, and spermiation. All of these steps of spermatogenesis take place during Stages VII and VIII indicating that RA activity is only required during these two stages. (Figure adapted from [9]).



This observation strongly indicates that RA activity is only important during a very small window of spermatogenesis. It is, at this point, unclear if RA itself is regulated in a pulsatile manner across the spermatogenic cycle, or if RA is present throughout the cycle, but another level of control renders its presence inert until the cells within the tubule are ready to respond. It is likely that these two ideas are not mutually exclusive. In order to further tease this question apart, stage-specificity of relevant players associated with RA metabolism, signaling, transport, and storage must be assessed in order to determine how all of these processes could contribute to the hypothesized pulse of RA.

5.1. Retinoid Metabolism

If RA is only present during Stages VII and VIII, how is this phenomenon regulated? Is RA synthesis or degradation responsible for generating this pulse? Localization studies from mice (reviewed in [31]) have provided some clues. *Aldh1a1* has been observed in all Leydig and Sertoli cells [48]. *Aldh1a2* transcript is expressed in late spermatocytes and early spermatids but does not appear to be stage-specific [48] and a separate protein localization study has confirmed localization of ALDH1A2 to early spermatids but not late spermatocytes [49]. Finally, *Aldh1a3* mRNA was only detected at low levels in Leydig cells [48]. Another retinoid metabolizing gene that may be of biological significance is *Rdh11*, which was shown to have variable expression across the spermatogenic stages and is present in pachytene spermatocytes [50]. The retinoid degrading genes, *Cyp26a1*, *Cyp26b1*, and *Cyp26c1*, all localize to peritubular myoid cells, which form the outer layer of the seminiferous tubule, in all stages [48]. CYP26B1 enzyme has also been noted in the peritubular myoid cells [49].

Taken together, this localization data can provide a working hypothesis. If RA metabolism within the seminiferous tubule is, in fact, what is responsible for maintaining spermatogenesis, then logically, the enzymes responsible for either the synthesis or the degradation, or both, would be present in the testis in a stage-specific manner. This, however, is not the case [48,49]. It is possible that RAL availability is indirectly responsible for generating this hypothesized pulse of RA, as preliminary data suggests that *Rdh11* is present in a stage-specific manner [50]. Further investigation needs to be performed to determine if protein localization and enzymatic activity of the vitamin A metabolizing enzymes fluctuates with the cycle of the seminiferous epithelium in order to generate a cyclic RA pulse.

5.2. Retinoid Signaling

If, it turns out, RA metabolism is not—or only partially—responsible for the observed RA response, then another method of regulation must exist. One candidate may be the cell's ability to respond to RA. Again, localization studies have provided insight into whether there is stage-specificity associated with RA signaling molecules (reviewed in [31]). *Rara* and *Rxrb* transcript and protein were detected in Sertoli cells of all tubules, while *Rarb* and *Rxra* transcripts were localized to round spermatids, specifically in Stages VII and VIII [48]. A separate study in adult rats found that RARα is expressed in spermatids [51]. *Rarg* mRNA was detected at the periphery of all tubules, while *Rxrg* mRNA was localized to some round spermatids in a fraction of Stage VII and VIII tubules [48]. RARβ is expressed in Sertoli cells, while RXRα and RXRγ are expressed in the majority of germ cells [52]. If retinoid signaling is responsible for the stage-specific RA response, it is probably the result of signaling via

RARβ, RXRα, or RXRγ, or more likely, a combination of the three. Separate studies reported that *Rarb*-, *Rxrg*-, and *Rarb*/*Rxrg*-null animals have no phenotype associated with sterility [48,53,54]. It is possible that ablation of these genes caused a change in expression of the other RARs and RXRs to compensate for the loss. Moreover, these genes could be acting in a non-canonical manner. Regardless, more conclusive localization studies need to be conducted to flesh out currently reported inconsistencies.

5.3. Retinoid Transport and Storage

Retinoid transport and storage is another potential cause for variable RA levels across the spermatogenic cycle. *Stra6* is expressed specifically in Stages VI and VII [55], but no phenotype associated with fertility, or even aberrant spermatogenesis, was reported for *Stra6*-null animals [56]. The localization of *Crabp* genes, which encode for proteins responsible for cellular binding of retinoids, within the testis was also investigated. *Crabp1* localized to both undifferentiated and differentiating spermatogonia in the rat and the mouse [48,57], while CRABP2 was localized to the testis in rats via immunoblotting, though more specific localization data were not pursued [58]. *Lrat* is expressed in round spermatids during Stages II-VI and *Rbp1* transcript was detected in Sertoli cells during stages X-XI [48]. The localization patterns of both *Lrat* and *Rbp1* support the hypothesis that stage-dependent transport and storage of retinoids plays a role in cyclic RA availability within the testis.

A definitive investigation of whether retinoid metabolism, signaling, transport, or storage—or a combination of these—is responsible for the observed stage-dependent response to RA during spermatogenesis is still required. Understanding how this response is generated will be pivotal in moving forward the fields of both spermatogenesis and retinoid biology.

6. Conclusions

RA plays key roles in neonatal and adult spermatogenesis. In the neonatal murine testis, it has been hypothesized as being responsible for establishing the spermatogenesis in an asynchronous manner [59]. It also plays a role in many aspects of adult spermatogenesis, being vital for spermatogonial differentiation, meiotic initiation, and reorganization of the BTB, as well as release of spermatozoa into the lumen of the seminiferous epithelium. Importantly, all of these processes occur during the same two stages of spermatogenesis: Stages VII and VIII. This strongly suggests that RA is only necessary during these two stages, and other stages progress in either the absence of RA or these cells temporarily lack the ability to respond to RA.

Because RA is known to play such a vital role in so many different aspects of mammalian biology, it is crucial that we understand the action and regulation of this integral biological molecule, as it can have important implications in many different disciplines. Hopefully this review will elucidate avenues of future research in not only the field of spermatogenesis, but also in retinoid biology and development.

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Author Contribution

T. Kent and M. Griswold conceived these ideas. T. Kent wrote the manuscript. T. Kent and M. Griswold edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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