Clinica Chimica Acta 497 (2019) 54–60

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

The roles of retinoic acid in the differentiation of spermatogonia and spermatogenic disorders

Xuan Li[a,1], Xiang-yang Long[b,1], Yuan-jie Xie[a], Xin Zeng[a], Xi Chen[a,*], Zhong-cheng Mo[a,*]

[a] Clinical Anatomy & Reproductive Medicine Application Institute, Department of Histology and Embryology, Hengyang Medical School, University of South China, Hengyang 421001, China

[b] Department of Urology, The Second Affiliated Hospital, University of South China, Hengyang 421001, China

ARTICLE INFO

Keywords:
C-Kit
RAR
Retinoic acid
Spermatogenesis
Stra8

ABSTRACT

Male fertility depends on the regulatory balance between germ cell self-renewal and differentiation, and the spatial and temporal patterns of this balance must be maintained throughout the life cycle. Retinoic acid and its receptors are important factors in spermatogenesis. Spermatogonia cells can self-proliferate and differentiate and have unique meiotic capabilities; they halve their genetic material and produce monomorphic sperm to pass genetic material to the next generation. A number of studies have found that the spermatogenesis process is halted in animals with vitamin A deficiency and that most germ cells are degraded, but they tend to recover after treatment with RA or vitamin A. This literature review discusses our understanding of how RA regulates sperm cell differentiation and meiosis and also reviews the functional information and details of RA.

1. Introduction

Retinoic acid (RA) is an active intermediate product of the metabolism of vitamin A. Studies have shown that vitamin A deficiency (VAD) induces the gradual loss of rat germ cells, leaving only the Sertoli cells and undifferentiated spermatogonia in the seminiferous tubules. However, spermatogenesis in rats with VAD can be restored after an RA injection [1]. This phenomenon proves that RA is an active factor that induces spermatogenesis. The synthesis, transportation, and degradation of RA are strictly regulated in different tissues. The in vivo metabolic properties of RA include the absorption of precursors, the storage of retinol esters, the oxidation of esters to RA, and the degradation of RA into inactive metabolites. The conversion of vitamin A to RA requires two successive oxidation steps. These two oxidation steps are sequentially catalyzed by alcohol dehydrogenase (ADH) and retinaldehyde dehydrogenase (RALDH) [2]. RALDHs include the three isoenzymes ALDH1A1, ALDH1A2, and ALDH1A3. Among them, ALDH1A2 is the most important isozyme in the biosynthesis of RA [2,3]. In mouse testis, ALDH1A2 is distributed in the germ cells of the seminiferous tubules, while ALDH1A1 and ALDH1A3 are distributed in Leydig cells and Sertoli cells [4]. The levels of ALDH1A1 and ALDH1A3 in the human testes of sterile males were not found to be significantly different from the control population, but the ALDH1A2 protein level in the testes of infertile men was significantly reduced and was closely related to the germ cell population [3].

Once RA enters the target cell, it has one of two fates: it can bind to the retinoic acid receptor and induce a series of cellular responses, or it can be degraded by the cytochrome P450 family of enzymes(CYP26A1, CYP26B1, and CYP26C1) [2,4]. There is a precise balance between the synthesis and degradation of RA, the degrading enzyme CYP26B1 plays an important role in maintaining the balance of RA [5]. Retinol and a small amount of RA are transported to the cell by retinol binding protein 4 (RBP4), where Retinol binds to STRA6 to enter the cytoplasm, is converted to RA, and then binds retinoic acid binding proteins (CRABPs), which translocate it to the nucleus. RA then binds to its high-affinity receptor and acts on the RA response element of the target gene promoter and regulates the transcription of related genes [6,7]. The main source of RA in the testis is from its own synthesis, which is especially predominant in Sertoli cells (SCs). The degradation of RA in the testes most likely occurs in SCs and Leydig cells [8]. Testosterone can promote the expression of the gene that encodes RA synthetase and can inhibit the expression of a gene that encodes an RA-degrading enzyme, thereby controlling RA biogenesis. RA directly controls sperm differentiation, thus determining spermatogenesis in seasonal mammals [9].

The process of spermatogenesis originates in spermatogonia stem cells (SSCs) [10]. Mouse SSCs originate from a single-cell state called a single (As) spermatogonia, which upon division gives rise to two paired

* Corresponding authors.
E-mail addresses: chenxi_submit@163.com (X. Chen), zhchmo@hotmail.com (Z.-c. Mo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cca.2019.07.013
Received 10 May 2019; Received in revised form 20 June 2019; Accepted 10 July 2019
Available online 11 July 2019
0009-8981/

A (Ap) spermatogonia and then to a chain of 4–32 aligned (Aal) spermatogonia. As, Ap, and Aal are referred to as undifferentiated spermatogonia, and they all retain stem cell properties. Undifferentiated spermatogonia are uniformly distributed in the seminiferous tubules and exit from the stem cell pool on a regular basis. After undergoing nonmitotic differentiation, they differentiate into type A1 spermatogonia [11–13]. The differentiation pathway begins, and the spermatogonia then undergo five mitotic transformations to A2, A3, A4, intermediate (Int) and B spermatogonia, and they then become preleptotene spermatocytes and initiate meiosis, which can be determined with commonly used markers such as RA-induced Stra8, c-Kit, and so forth. The transformation from type A1 to type B spermatogonia is called "differentiated spermatogonia" and results in the loss of the cell's ability to self-renew [14]. RA is a potential regulator of Aal to A1 transition in rats and mice. In the absence of vitamin A, the vast majority of germ cells are halted at the stage of undifferentiated spermatogonia [12].

During the fetal period, RA is produced in the mesonephros of the fetal testis. However, the RA metabolizing enzyme CYP26B1, which is produced by SCs and Leydig cells, can degrade RA, thereby preventing meiosis. After birth, SSCs resume proliferation and become spermatogonia [15]. The meiosis of primary spermatocytes begins in prepuberty. Under the action of RA, the two meiotic divisions that are involved in spermatogenesis proceed without interruption, resulting in the formation of haploid spermatids, which undergo morphological changes and eventually become spermatozoa [11], and this entire process occurs in the spermatogenic epithelium (SE) of the testis (Fig. 1).

2. RA is essential for spermatogenesis

Studies have found that RA can affect human sperm metabolism and antioxidant defense systems. One of the most important causes of sperm dysfunction is the disruption of the balance of the antioxidant defense system in the male reproductive tract. This imbalance has a serious impact on normal fertilization, capacitation, exercise and acrosome reaction [16]. Mammalian spermatogenesis begins with diploid spermatogonia and ends with haploid spermatozoa. The process involves four key transformations: spermatogonia differentiation, meiotic initiation, initiation of spermatid elongation, and release of spermatozoa [12,17]. Measurements indicate that RA undergoes periodic changes, where RA levels are lower before the four transitions, increased during conversion and keep increasing thereafter [12]. The periodic RA-Stra8 signal interacts with periodic germ cells to regulate two different cellular process-specific responses: spermatogonia differentiation and meiotic initiation [18], and RA may also be involved in the regulation of the latter two transition periods of spermatogenesis. RA inhibits the genes that are involved in promoting the proliferation of undifferentiated spermatogonia, including mSSCs and progenitor spermatogonia, while RA also activates the genes that are involved in spermatogonia differentiation and meiotic initiation and progression [19]. Transplantation analysis confirmed a significant increase in the number of testicular SSCs in VAD animals. In contrast, no change in the number of SSCs was detected after exposure to excess RA. This indicates that RA deficiency results in an increase in the proportion of undifferentiated spermatogonia that remain in the SSC state [20].

The four key transitions periods of spermatogenesis are precisely coordinated in time and space, and they are closely linked physically and temporally and have an 8.6 days periodicity in mice [21]. Studies have shown that there are 12 stages in the spermatogenic epithelial cycle called the spermatogenic phase I to XII, and the four key transitions of spermatogenesis occur in stages VII and VIII [22]. In mice, the

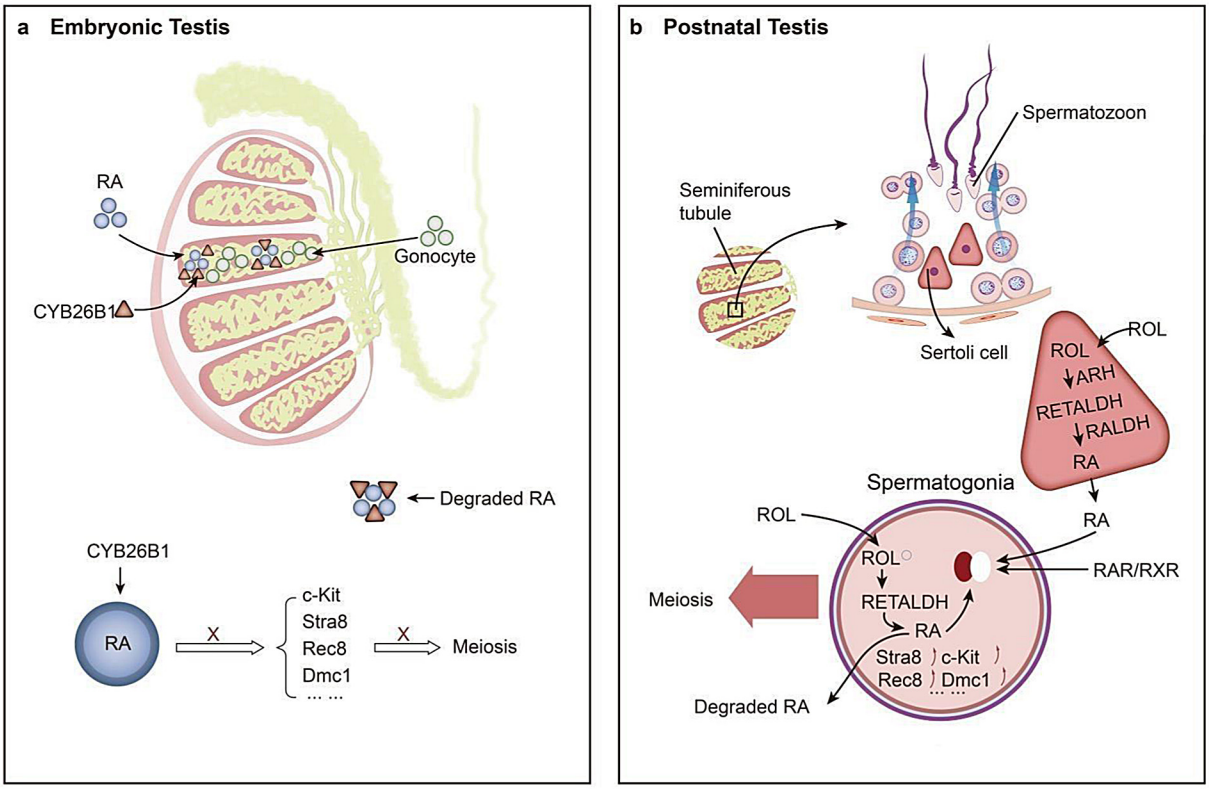

Fig. 1. The effect of RA on male germ cells differs between prenatal and postpartum. a). In the embryonic testis, although a certain amount of RA (blue circle) is present, the RA metabolic enzyme CYP26B1 (red triangle) can degrade the RA. Inhibition of expression of downstream genes such as c-Kit, Stra8, Rec8, Dmc1, etc., thereby inhibiting spermatogonia differentiation and meiosis processes. b). In postpartum testes, Retinol (ROL) is internalized by Sertoli cells or spermatogonia, first converted to retinal (RETALDH) by alcohol dehydrogenase (ADH), and then catalyzed by retinal dehydrogenase (RALDH) to RA. RA can enter spermatogonia to bind to RAR/RXR dimers, up-regulate downstream gene expression and initiate meiosis. RA will then be degraded by CYP26. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

process of spermatogenesis takes approximately 35 days, allowing the diploid spermatogonia to produce haploid sperm, which is equivalent to four rounds of the seminiferous epithelium cycle [23]. In the normal testis, due to their differentiation in stages VII and VIII, spermatogonia express the oncogene c-Kit and enter the mitotic phase, where they eventually develop into type B spermatogonia, become preleptotene spermatocytes and initiate meiosis, and then generate leptotene spermatocytes [18].

2.1. RA promotes the differentiation of spermatogonia

Spermatogenesis begins with undifferentiated type A spermatogonia, undergoes a periodic Aaligned-to-A1 transition, and results in differentiated spermatogonia [12]. The spermatogonia lose their ability to self-renew during differentiation [24]. Long-term vitamin A deprivation leads to an impaired Aaligned-to-A1 transition in spermatogonia or to a stagnation in the preleptotene spermatocyte stage [15,25]. Most germ cells (GCs) degenerate, leaving spermatogonia type A in the testis of VAD mice, and some preleptotene spermatids may also be present [26]. After an injection of vitamin A or RA, the arrested spermatogonia begin to differentiate [18,27]. Spermatogenesis synchronously restarts throughout the testis, and this synchronization may continue for several months [26,28].

Just before spermatogonia differentiation and meiosis occur, the endogenous RA levels in the seminiferous epithelium during stages I-VI are low because of very low or undetectable levels of RA synthase. It is known that the only transcript encoding the RA uptake protein Stra6 is present in SCs, and the highest level of RA is detected in stages VII-IX, which is consistent with the requirement of RA for spermatogonia differentiation. The ALDH1A2 transcript was also observed in pachytene spermatocytes in stages VII-XII, indicating that the differentiated GCs are synthesizing RA during these stages. After the differentiation of spermatogonia, the highest level of CYP26A1 mRNA can be detected in stages VIII-XI, when it promotes the degradation of RA. Evidence suggests that the pulsed concentration gradient of RA on neonatal testicular tubules drives spermatogonia differentiation in a precise and repetitive manner so that sperm is continuously produced in the adult testis [4,29,30].

2.2. RA accelerates the initiation of meiosis

In mammals, the signal of RA is mediated by its target gene Stra8, which is necessary for the initiation and progression of meiosis [19]. RA binds to and activates nuclear RA receptors and controls the expression of its downstream target genes, which is required to induce meiosis in spermatocytes. Some germ cells in VAD mice also arrest before meiosis. After supplementation with vitamin A or RA, stagnant preleptotene spermatocytes begin meiosis [12]. The premeiotic transition is coordinated by the RA from the SCs. Once the male GCs enter meiosis, the pachytene spermatocytes produce RA to coordinate the two meiotic transitions. The two sides are coordinated in time and space to ensure the production of large amounts of sperm [12].

Considering the two meiotic transitions - the activation of spermatid extension and sperm release – are consistent with high RA levels, we believe that RA may also regulate these transitions. Knockout of RA receptors (RARs) in mice results in various modest defects in meiosis and post-meiotic aspects of spermatogenesis (including sperm release) [31,32]. Studies have shown that RA can promote the initiation of sperm cell elongation, and RA can also induce sperm release [12]. Despite these findings, the specific steps by which RA may regulate meiotic progression or post-meiotic differentiation have not yet been determined. Furthermore, its role in triggering meiosis is still in dispute.

2.3. Injection of exogenous RA lead to synchronous spermatogenesis

Recent reports show that the first wave of spermatogenesis is actually asynchronous and occurs faster than the subsequent waves [33]. In mice, the first wave begins a few days after birth, when the SSCs turn into differentiated A spermatogonia, divide at 8 days postpartum (dpp) and subsequently produce preleptotene spermatocytes [33–35]. In normal mice, sperm production is constant due to the temporal and spatial stability of spermatogenesis [36]. The first differentiation step of spermatogonia occurs from the RA response along the seminiferous tubules in the neonatal testis when the cell contains a complete RA signaling mechanism and expresses β-galactosidase [33,37].

In the absence of RA, undifferentiated spermatogonia are arrested at G0. There is evidence that exposure to RA may increase the number of testicular SCs after birth [37]. WIN 18,446 is a potent inhibitor of retinaldehyde dehydrogenase (RALDH) and can lead to decreased levels of RA in the testes of male mice, blocking male GC differentiation and producing testes filled with undifferentiated spermatogonia. As a result, the normal 14 complete stages in the seminiferous tubules are disrupted and instead only contain 3 to 4 stages [11]. However, at any one time point, the frequency of the spermatogenic epithelial cell cycle contained in the testes of the treated animals was much higher than that of normal individuals. At these stages with frequency anomalies, significant “cut off waves” were generated, and the spermatogenesis of these animals was described as synchronous. Recent studies have shown that testicular exposure to an exogenous RA environment in prepubertal mice with sufficient vitamin A drives the entire undifferentiated population into a differentiation pathway, leading to synchronous spermatogenesis [11]. After synchronization, some of the differentiated spermatogonia subtypes may be skipped with plasticity, and the undifferentiated A spermatogonia are directly transformed to A2 or A1 spermatogonia, followed by division into A3 [37].

Exposure of a vitamin A sufficient adult testicle to a higher concentration of RA does not result in simultaneous spermatogenesis, but instead induces apoptosis in a subpopulation of spermatogonia. The exposure of neonatal testes to high concentrations of RA changes the GC differentiation and meiotic markers (Stra8, c-Kit, Sycp3, and Rec8) that are observed in these cells and also increases the number of STRA8 and SYCP3 immunoreactive cells. In addition, RA exposure of the adult testes resulted in increased expression of the RA-degrading enzyme CYP26A1. Therefore, synchronous spermatogenesis occurs only after neonatal RA exposure [38].

2.4. The interaction among RA, SCs, and spermatogonia

SCs are the only somatic cell types that physically contact spermatogenic cells in vivo [19]. They not only provide physical and nutritional support, but they also secrete factors that are critical in the complex developmental processes of GC proliferation and differentiation, which involve retinoic acid synthesis and blood-testis barrier biogenesis [39]. Throughout the embryonic period, SCs undergo extensive mitotic activity to establish a guard cell population of progenitor cells. From one week after birth, the SC proliferation rate in rats rapidly decreases until 15 days postpartum into the quiescent phase of mitosis. After the SCs stop proliferating, a tight junction is formed between the adjacent SCs, and a testis and blood barriers is generated for a sealing function. This barrier divides the seminiferous epithelium into the basal and apical compartments. This SC differentiation process is essential for male fertility. In addition, this process is affected by the regulation of RA signaling [23]. The SC in the seminiferous tubule that directly interacts with SSCs and controls their proliferation and differentiation by secreting specific factors [40]. In vitro experiments demonstrated that RA alone was sufficient to induce mSSCs into primary spermatocytes and that the coculture of RA with SCs obtained from young mice formed a more efficient in vitro meiotic model. RA in combination with pup SC coculture also resulted in a higher induction

efficiency of 28% [19]. In early puberty, the RA required in the first spermatogenesis cycle depends on RALDH synthesis in SCs [13]. SCs play an important role in the process of spermatogenesis.

A very specific expression pattern of the genes that are involved in the synthesis and action of RA is observed in the SCs, which is necessary for SCs synthesis to induce spermatogonia differentiation during the first spermatogenic cycle [13]. In the first spermatogenesis cycle, SC-derived RA is required to induce the differentiation of spermatogonia, and it is essential for sperm production during the subsequent spermatogenic cycle [13,39]. SC can express multiple RA-related genes, which include the retinaldehyde dehydrogenase genes (ALDH1A1, ALDH1A2, ALDH1A3) and the nuclear receptors RAR-A, RXR-A and RXR-B. The investigation revealed that all ALDH1A1 genes and the corresponding nuclear receptors for RA are expressed in SC; however, ALDH1A2 can only be detected in germ cells before puberty is completed. Their different expression profiles indicate that the regulation of RA synthesis in SCs is complex and that RA may act directly on immature SCs [23].

SCs can strictly regulate the local uptake, oxidation, signaling and degradation of retinoid in the testes [41].The metabolic barrier regulates exogenous RA in the testes and results in the delivery of RA to the GCs in a temporal and spatial order; this, in turn, controls the differentiation of spermatogonia and their eventual entrance into meiosis [26]. Testicular SCs can produce RA and regulate the premeiotic transition [12]. RAR-A in the SCs can regulate tight junctional complexes between Sertoli cells, and at the proper time, allows the preleptotene spermatocytes to pass through the chamber and to pass through meiosis [12]. The testis is a tissue that is very resistant to exogenous RA [42]. Therefore, we observed that high doses of exogenous RA have a very limited effect when administered to vitamin-deficient adult males [26]. SCs ensure that the spermatogenesis of GCs can proceed properly despite their exposure high doses of RA and also ensures the self-renewal and proliferation capability of some spermatogonia cells. SCs also serve as a storage pool for vitamin A for the uptake of retinol, and they maintain a large reserve of retinol ester analogues to maintain the in vivo balance of RA [43]. Together, these results indicate that complete RA signaling is required for the maturation and function of SCs. However, the mechanism by which RA simultaneously induces the differentiation of GCs and SCs in puberty remains unclear.

3. The role of RA nuclear receptors in spermatogenesis

The process of spermatogenesis is deficient in the testis of VAD rats by several major factors: failure of A2 sperm to form from A1 spermatogonia, delayed precursor cell development and dysplasia, and reduced spermatids [31]. Studies have demonstrated that certain receptors that are present in the early GCs of neonatal testes allow for the necessary components to respond to RA signaling pathways, including RA receptors and retinoid receptors [33]. RA is poorly transported in the serum and usually stores dietary vitamin A in the liver and binds to the plasma retinol-binding protein Rbp4 to form a complex that is transported to a target organ such as the testis. RA then binds to two families of intracellular receptors [23]. RA signaling is mediated by the two receptors retinoic acid receptor (RAR) and retinoid X receptor (RXR), both of which have three major subtypes: A, B, G. These receptors belong to the superfamily of nuclear receptors and act as ligand-dependent transcription factors in the nucleus [44]. Studies have shown that male mutant mice in which the RA receptors of the Leydig cells are inactivated in are unable to perform RA signaling. In these mice, the apoptosis of the pachytene spermatocytes in the testis was increased, the mature germ cells were deleted, the number of macrophages in the interstitium was increased, and the epididymis also showed an abnormal phenotype [45].Therefore, RA signaling is necessary for male fertility.

RAR-A in SCs may establish RA signaling by activating the expression of the gene Mafb, and the RA that is produced by the SCs activates RAR-A in an autocrine manner, which drives the first wave of spermatogenesis and results in the transition from Aal to A1 [13]. All RARs can be detected in specific cell types in the seminiferous epithelium [46]. RARs can be activated by both 9-cis retinoic acid and 11-all-trans retinoic acid, and RXRs are only activated by 9-cis retinoic acid [44,47]. Studies have shown that during retinol- and RA-induced spermatogenesis, some of the RAR and RXR levels are upregulated in the testis of VAD rats [48]. RA binds to and activates its nuclear receptor and controls the expression of downstream target genes.

3.1. The response of spermatogonia to RA signaling depends on the RAR/RXR heterodimer

Due to the complexity of retinoid signaling, its receptor may form different heterodimers. These dimers can bind to different reaction elements [47]. RAR can bind to RXR to form an RAR/RXR heterodimer, which then binds retinoic acid responsive DNA elements (RAREs) that are located in the genome regulatory region and modulate RARE-controlled RA-dependent transcriptional genes [44]. This process requires the recruitment of co-receptors and gene cofactors for gene induction, or the recruitment of co-repressors to inhibit transcriptional activity [15]. Inactivation of RAR or RXR blocks the RA signaling pathway and leads to male infertility. In RAR-A$^{-/-}$ mutant and RXR-B$^{-/-}$ mutant testes, mature spermatozoa do not appear in the seminiferous epithelium, and abnormal spermatozoa are indicated in the epithelium in stages IX and X of the spermatogenic cycle [49]. Further investigations should be performed to determine how RA-activated RAR/RXR heterodimers drive sperm cell release and how this process can be applied to new male contraceptive regimens.

3.2. Injury of RA receptor will lead to abnormal phenotype of the testis

RAR-A and RXR-B are the only functional retinoid receptors in Sertoli cells [23]. Because the mutation causes its testes to produce scarce and abnormal sperm, RAR-A$^{-/-}$ mutants and RXR-B$^{-/-}$ mutant male animals all show infertility [23,44]. RXR-B autonomously controls lipid metabolism in supporting cells [49]. Knocking out the RXR-B gene in the mouse testis leads to impaired sperm cell maturation and delays sperm activity [12]. RAR-A is an essential medium for RA signal transduction in the testes; RAR-A induces the expression of Nrg1 and Nrg3 (neuregulin) and promotes the proliferation of spermatogonia [50]. RAR-A controls the first wave of spermatogonia differentiation in SCs; the first wave of spermatogenesis in RAR-A$^{-/-}$ mutants was abnormal due to the delayed differentiation of spermatogonia, which was characterized by the delayed expression of the target gene Stra8 and reduced proliferation of spermatogonia. The second wave of spermatogenesis was also delayed in these mutants, and the development of preleptotene and leptotene spermatocytes was suspended [31]. The oscillatory activity of SCs and the integrity of the seminiferous epithelium depend on RAR-A; similar to VAD rats, RAR-A knockout mice are unable to maintain a normal spermatogenic cycle [23]. Functional disruption of RAR-A in SCs may lead to the arrest of preleptotene spermatocytes, and disruption of RAR-A in germ cells may cause the blockade of A1 spermatogonia in the VAD model [12]. RAR-A-deficient animals have higher a neonatal mortality rate and exhibit a lethal phenotype in males. The GCs in the testes of surviving male mice are depleted and vacuolated [8]. RAR-A expressed in germ cells can also interact with Sertoli cells, regulate the integrity of the blood testis barrier, and coordinate the development and spatial arrangement of mouse testicular GCs [51].

RAR-B$^{-/-}$ male animals are infertile, but their genital and reproductive tracts remain histologically normal throughout life. Therefore, RAR-B seems to have no effect on the homeostasis of the seminiferous epithelium [46]. RAR-G plays a role in spermatogonia to direct A1 differentiation [23]. RAR-G can control the ability of spermatogonia cells to respond to RA [52]. Since part of spermatogonia do

not express RAR-G, spermatogonia can maintain the differentiation and self-renewal capabilities even when exposed to high doses of RA [11]. Inactivation of RAR-G did not affect the expression of the spermatogonia differentiation markers Kit and Stra8. RAR-A can compensate for the loss of RAR-G function during prepubertal spermatogenesis. Therefore, the RAR-G$^{-/-}$ mutation does not affect meiosis or the synchronization of cell differentiation during spermatogenesis [46]. The above revealed finding the importance of RAR-A and RXRB in the spermatogenic cycle.

A low dose of the RAR antagonist BMS-189453 can destroy the RA conduction system so that process of the spermatogenesis is destroyed. Similar to VAD and RAR-A knockout mice, there was a loss of GCs in the testes, along with an abnormal sperm arrangement and failure of sperm release into lumen. Antagonist-induced infertility is reversible, and since the spermatozoa can fully recovered after a prolonged inhibition, this antagonist can be used to develop nonsteroidal male contraceptives [53,54].

RAR-A controls the first round of spermatogonia differentiation in GCs. RA promotes GC differentiation through paracrine modes during prepubertal spermatogenesis. Therefore, abolishing RA synthesis activity in GCs will impair the differentiation of spermatogonia, and treatment of these mutants with the RAR-A agonist BMS 753 can restore the capacity for spermatogonia differentiation [23]. Studies have shown that pan-RAR antagonists can suppress the expression of the target gene STRA8 and prevent spermatogonia from entering meiosis [55,56]. Two pan-RAR antagonists including BMS-204493 and AGN 193109 have been shown to inhibit meiosis [55]. In contrast, RARs-specific agonists can induce the expression of Stra8 in embryonic testis [15,56]. The destruction of key RAR-A signaling pathways leads to testicular degeneration, including the vacuolization of Sertoli cells, detachment of spermatocytes and spermatids, and the eventual reduction of sperm production, leading to male sterility [57].

4. RA regulates the expression of its downstream target genes during spermatogenesis

RA directs differentiation by altering the gene expression of spermatogonia [18]. The role of RA in GC differentiation has also been confirmed because of the increase in the genetic markers that are associated with spermatogonia differentiation in RA treated neonatal testes such as Proto-oncogene tyrosine kinase c-Kit (Kit) and Retinoic acid 8 (Stra8) and decreased expression of Pou5f1, which is an undifferentiated GC-related marker [33,58].

RA can not only promote the differentiation of spermatogonia by upregulating the c-Kit gene but can also upregulate the Stra8 gene to help initiate meiosis and upregulate the genes Rec8, Dmc1, Prdm9, Sycp3, and so on to promote meiosis; in addition RA can downregulate beneficial mSSC self-renewal genes, such as Oct4, Plzf, Lin28a, and so on [19].

4.1. RA is a key upstream regulator that controls the expression of c-kit

The proto-oncogene c-Kit is a growth factor receptor with tyrosine kinase activity, also called Kit, and it maintains the balance between self-renewal and differentiation in SSCs. RA activates the RAR/RXR heterodimer to increase the expression of the SALL4A transcription factor in spermatogonia. The SALL4A-encoded transcription factor is known to regulate c-Kit expression, which increases RA to promote the expression of the c-Kit tyrosine kinase receptor and induce the differentiation of spermatogonia [59]. The bone morphogenetic protein 4 (BMP4) receptors BMPR1A and BMPRII are present in spermatogonia, spermatocytes and round spermatids. Although exogenous BMP4 alone is not sufficient to induce the expression of Stra8 and c-Kit (two marker genes for spermatogonia differentiation), significant synergistic effects of BMP4 and RA have been observed. BMP4 can exert an autocrine effect and cooperate with RA to induce the differentiation of testicular spermatogonia in vivo [60]. The RXR and RAR dimers can bind to regulatory regions located in the Sall4 gene, thereby driving the spermatogonia differentiation process. The transcription factor encoded by the gene Sall4 regulates the expression of c-Kit, which is often dysregulated in testicular cancer [61]. This dynamic pattern of transcription and translation is exhibited by c-Kit during the entire SSCs differentiation process, and RA plays a key role as an upstream regulator of the expression of c-Kit [62].

Studies have shown that mRNAs for c-Kit are present in undifferentiated spermatogonia but not translated. However, RA can induce efficient translation of c-Kit mRNA through the PI3K / AKT / mTOR signaling pathway, thereby promoting differentiation of spermatogonia. In normally differentiated spermatogonia, the protein of c-Kit should have been well translated. However, the translation of c-Kit protein is poor under VAD conditions [63]. RA promotes the expression of the gene c-Kit, which is consistent with the transformation of Aal spermatogonia into the first generation of differentiated A1 spermatogonia, and it also allows the maintenance of a differentiated spermatogonia state [46]. Stimulation with RA induced the quantitative expression of c-Kit as a pattern of increased and decreased waveform changes that were similar to the development of male GCs in vivo [64]. RA injection induces the expression of precocious c-Kit in spermatogonia [18]. Some studies have confirmed that exogenous RA cannot directly stimulate c-Kit expression. RA indirectly affects the expression of c-Kit in male GCs, and some unknown factors of testicular somatic cells may be involved [64].

4.2. Periodic RA-Stra8 signaling regulates meiotic initiation

RA induced the expression of the meiotic marker Stra8, followed by expression of early meiotic markers Sycp3 and Dmc1 [65]. High levels of Stra8 mRNA transcription were first observed at 6-dpp in several type A and type B spermatogonia, preleptotene spermatocytes, and leptotene spermatocytes, and this was followed by peaks at 10-dpp and then at 14-dpp. Furthermore, the levels at 18-dpp began to drop significantly. This is consistent with the occurrence of meiosis I [15,26]. In the adult testis, the highest levels of Stra8 mRNA and protein were observed in the seminiferous epithelium stages VI to VIII [26]. The upregulation of Stra8 expression by RA is critical for spermatogenesis. In the embryonic testis, the lack of Stra8 expression is mainly due to the degradation of RA by CYP26B1 [66]. Without the function of the gene Stra8, meiosis is blocked.

The gene Stra8 controls mitosis in GCs and meiosis in GCs and also acts as a meiotic gatekeeper. The promoter region of Stra8 contains RARE, which can bind to the RAR/RXR heterodimer and make itself a direct target for RA [11]. Stra8 is required for the initiation of meiosis in males, and Stra8 deficient GCs arrest before meiosis [18]. RA synthesized from premeiotic spermatocytes, and it autonomously induces meiotic initiation by controlling the RAR-dependent expression of Stra8 [13]. Histological differences were found between wild-type testes and Stra8 deficient testis at 10-dpp and 15-dpp. The population of spermatogenic cells in the wild-type testis at 10-dpp began at meiosis and progressed to leptotene spermatocytes. This is the first phase of the premeiotic division. These cells were subsequently converted to zygotene and progressed to pachytene spermatocytes of meiotic prophase at 15-dpp. In contrast, leptotene, zygotene, or pachytene spermatocytes were also absent in the knockout Stra8 testis. These finding suggest that the transformation of spermatogenic cells from preleptotene to leptotene requires the participation of Stra8 to thus enter the pre-meiotic phase [67].

What is the reason for preventing GCs from entering the meiosis process? Since Stra8-deficient testicular germ cells do not encode the topoisomerase Spo11 that is required for DNA double-strand breaks (DSBs) and do not encode the recombinase Dmc1, which plays a role in meiotic DSBs repair [68,69], meiotic recombination cannot be performed in these cells. SYCP3 (synaptonemal complex protein) and REC8

(meiosis-specific cohesin) modify the length of chromosomes in most wild-type spermatogenic cells [70,71], resulting in the presence of synaptic and meiotic adhesin complexes, respectively. In contrast, in Stra8 deficient spermatogenic cells, the SYCP3 and REC8 proteins are not loaded on chromosomes, although they are present. Therefore, Stra8 deficient spermatogenic cells do not undergo chromosomal aggregation and association. Stra8-defective preleptotene spermatocytes can undergo DNA replication but cannot enter into the pre-meiotic phase [67]. In Stra8 knockout mouse testes, the expression levels of Prdm9 (highly expressed in preleptonema and early leptonema) and Msh5 (highly expressed at the early preleptotene to zygotene stages) were significantly reduced, resulting in meiotic arrest and DNA double-strand breaks [66]. In conclusion, Stra8 is required for spermatogonia to undergo morphological changes, and RA signaling is required to induce GCs to express Stra8.

5. RA and male infertility

According to the World Health Organization, male infertility accounts for about 40% of all infertility. And the cause of most male infertility is still unclear, only manifested as spermatogenic disorders [72]. In recent years, in vitro fertilization technology has brought hope to infertility patients, but failed to solve the primary cause of the disease. Therefore, it is necessary to fully understand male infertility and actively carry out effective treatment methods. The important role of RA in spermatogenesis has been confirmed above. However, the relationship between RA and human infertility is unclear.

With the improvement of life quality, it is rare that vitamin A deficiency caused by nutrition. It is not clear whether deficiency of RA will contribute to infertility in men. However, recent case-control studies have shown that injury of RA signal pathway is associated with male infertility. ALDH1A2 is the most important isozyme in the synthesis of RA. Compared to normal males, testicular biopsy results showed that levels of ALDH1A2 protein were significantly reduced in testicular tissues of infertile men. Moreover, the level of ALDH1A2 is closely related to the number of GCs [3]. A recent clinical trial have shown improvement in sperm counts of 19 infertile male after being orally treated with RA [73]. It is necessary to further study the treatment mechanism of RA for human infertility.

Varicocele is one of the most common cause of male infertility. The incidence of varicocele in male population is 10% to 15%. Varicoceles are present in more than 35% of men with primary infertility, and in up to 80% of men with secondary infertility. According to a study found that RAR-A exists and localizes in human sperm cells, and has demonstrated a decrease in the molecular expression level of RAR-A protein in varicocele samples [74]. Compared with normal human semen, reactive oxygen species (ROS) in the semen of patients with varicocele significantly increased, and ROS began to decrease after using RA [16]. It is proved that RA has a potential therapeutic effect on oxidative stress in male infertility.

Cryptorchidism is a common congenital malformation in the male reproductive system with an incidence of up to 3–4% [75]. The GCs (especially haploid sperm) in the testes of cryptorchidism patients significantly reduced or even completely disappeared, thus affecting male fertility. Some researchers have co-cultured SSCs extracted from human cryptorchidism by RA and stem cell factor (SCF), and results have showed that these SSCs began to differentiate, meiosis, and formed fertility haploid sperm [76]. RA is a potential treatment for male infertility with low toxic side effects, and may improve infertility caused by various diseases.

6. Conclusion and prospect

Male infertility is significantly increased due to decreased sperm function and increased sperm count. The assessment and treatment of male infertility is becoming increasingly important. RA controls a variety of life activities such as normal fetal development, cell proliferation and differentiation, and apoptosis. Although RA plays a central role in spermatogenesis, the driving mechanism of RA is still largely unknown. Treatment of stem cells with RA and the protein synthesis inhibitor cycloheximide (CHX) can identify potential target genes for RA. Although we cannot predict how many downstream target genes are regulated by RA, it is believed that this number should be based on a combination of RA-regulated genes and RA + CHX-regulated genes, including 1041 upregulated genes and 1768 downregulated genes [19]. This quantitation requires long-term research. Certain environmental contaminants may interfere with endocrine secretion, primarily through the nuclear receptor signaling pathways that affect the mammalian reproductive system. For example, organotin compounds are agonists of the RXR subtypes that induce transcription of a target gene upon binding, resulting in changes in the expression of some proteins that are related to processes such as mitochondrial and cellular dysfunction. It also inhibits the catalytic activity of human aromatase and other steroidogenic enzymes and affects sexual development in rats [8].

The drop in testicular sperm count remains worrisome because the decline in the number or concentration of sperm increases the incidence of male infertility. Many factors lead to infertility and/or dysfunctional spermatogenesis in males, and most of the genetic mechanisms remain unclear [77]. Although the role of RA in guiding the differentiation of testicular spermatogonia has been clarified, the mechanism of the downstream activation of RA is largely ambiguous. The low cost and low toxicity of RA can provide tremendous advantages for clinical treatment. Elucidating the mechanisms by which RA participates in spermatogenesis will help in the treatment of some dysfunctions of spermatogenesis that are caused by defects in the RA signaling pathway.

Author contributions

XC and ZCM participated in the design, conceived the idea and revised the manuscript. XL and XYL drafted the manuscript. XZ participated in the revision of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

All authors declare no competing interests.

Acknowledgments

This work was supported by the Natural Sciences Foundation of Hunan Province (2018JJ2344), the Key Lab for Clinical Anatomy & Reproductive Medicine of Hengyang City (2017KJ182), the College Students' Research Learning and Innovative Experiment Plan in University of South China (2017XJYZ049, 2017XJYZ047) and the Chuanshan Talents Project in University of South China.

References

[1] M.D. Griswold, et al., Function of vitamin A in normal and synchronized seminiferous tubules, Ann. N. Y. Acad. Sci. 564 (1989) 154–172.
[2] G. Duester, Retinoic acid synthesis and signaling during early organogenesis, Cell 134 (6) (2008) 921–931.
[3] J.K. Amory, et al., Levels of the retinoic acid synthesizing enzyme aldehyde dehydrogenase-1A2 are lower in testicular tissue from men with infertility, Fertil. Steril. 101 (4) (2014) 960–966.
[4] N. Vernet, et al., Retinoic acid metabolism and signaling pathways in the adult and developing mouse testis, Endocrinology 147 (1) (2006) 96–110.
[5] K. Kashimada, et al., Antagonistic regulation of Cyp26b1 by transcription factors SOX9/SF1 and FOXL2 during gonadal development in mice, FASEB J. 25 (10) (2011) 3561–3569.
[6] M. Kelly, et al., Transport of vitamin A across blood-tissue barriers is facilitated by STRA6, FASEB J. 30 (8) (2016) 2985–2995.
[7] K.B. Laursen, et al., An alternative retinoic acid-responsive Stra6 promoter regulated in response to retinol deficiency, J. Biol. Chem. 290 (7) (2015) 4356–4366.
[8] D. Macejova, L. Toporova, J. Brtko, The role of retinoic acid receptors and their

cognate ligands in reproduction in a context of triorganotin based endocrine disrupting chemicals, Endocr. Regul. 50 (3) (2016) 154–164.
[9] Y.J. Wang, et al., Testosterone-retinoic acid signaling directs spermatogonial differentiation and seasonal spermatogenesis in the Plateau pika (Ochotona curzoniae), Theriogenology 123 (2019) 74–82.
[10] T. Lord, M.J. Oatley, J.M. Oatley, Testicular architecture is critical for mediation of retinoic acid responsiveness by undifferentiated spermatogonial subtypes in the mouse, Stem Cell Reports 10 (2) (2018) 538–552.
[11] M. Teletin, et al., Roles of retinoic acid in germ cell differentiation, Curr. Top. Dev. Biol. 125 (2017) 191–225.
[12] T. Endo, et al., Periodic production of retinoic acid by meiotic and somatic cells coordinates four transitions in mouse spermatogenesis, Proc. Natl. Acad. Sci. U. S. A. 114 (47) (2017) E10132-E10141.
[13] M. Raverdeau, et al., Retinoic acid induces Sertoli cell paracrine signals for spermatogonia differentiation but cell autonomously drives spermatocyte meiosis, Proc. Natl. Acad. Sci. U. S. A. 109 (41) (2012) 16582–16587.
[14] M. Teletin, et al., Two functionally redundant sources of retinoic acid secure spermatogonia differentiation in the seminiferous epithelium, Development 146 (1) (2019).
[15] M.D. Griswold, et al., Initiating meiosis: the case for retinoic acid, Biol. Reprod. 86 (2) (2012) 35.
[16] R. Malivindi, et al., Influence of all-trans retinoic acid on sperm metabolism and oxidative stress: its involvement in the physiopathology of varicocele-associated male infertility, J. Cell. Physiol. 233 (12) (2018) 9526–9537.
[17] F. Chan, et al., Functional and molecular features of the Id4+ germline stem cell population in mouse testes, Genes Dev. 28 (12) (2014) 1351–1362.
[18] T. Endo, et al., Periodic retinoic acid-STRA8 signaling intersects with periodic germ-cell competencies to regulate spermatogenesis, Proc. Natl. Acad. Sci. U. S. A. 112 (18) (2015) E2347–E2356.
[19] S. Wang, et al., Retinoic acid is sufficient for the in vitro induction of mouse spermatocytes, Stem Cell Rep. 7 (1) (2016) 80–94.
[20] K.S. Agrimson, et al., Retinoic acid deficiency leads to an increase in spermatogonial stem number in the neonatal mouse testis, but excess retinoic acid results in no change, Dev. Biol. 432 (2) (2017) 229–236.
[21] E.F. Oakberg, Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium, Am. J. Anat. 99 (3) (1956) 507–516.
[22] D.G. de Rooij, Proliferation and differentiation of spermatogonial stem cells, Reproduction 121 (3) (2001) 347–354.
[23] M. Mark, et al., Role of retinoic acid receptor (RAR) signaling in post-natal male germ cell differentiation, Biochim. Biophys. Acta 1849 (2) (2015) 84–93.
[24] T. Shinohara, et al., Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells, Proc. Natl. Acad. Sci. U. S. A. 97 (15) (2000) 8346–8351.
[25] A.M. van Pelt, D.G. de Rooij, Retinoic acid is able to reinitiate spermatogenesis in vitamin A-deficient rats and high replicate doses support the full development of spermatogenic cells, Endocrinology 128 (2) (1991) 697–704.
[26] Q. Zhou, et al., Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes, Biol. Reprod. 79 (1) (2008) 35–42.
[27] C. Morales, M.D. Griswold, Retinol-induced stage synchronization in seminiferous tubules of the rat, Endocrinology 121 (1) (1987) 432–434.
[28] J.E. Siiteri, et al., Testicular synchrony: evaluation and analysis of different protocols, Biol. Reprod. 46 (2) (1992) 284–289.
[29] C.A. Hogarth, M.D. Griswold, Retinoic acid regulation of male meiosis, Curr Opin Endocrinol Diabetes Obes 20 (3) (2013) 217–223.
[30] R. Sugimoto, Y. Nabeshima, S. Yoshida, Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of sertoli cells in mouse seminiferous epithelium, Mech. Dev. 128 (11 − 12) (2012) 610–624.
[31] S.S. Chung, et al., Retinoic acid receptor alpha is required for synchronization of spermatogenic cycles and its absence results in progressive breakdown of the spermatogenic process, Dev. Dyn. 230 (4) (2004) 754–766.
[32] S.S. Chung, X. Wang, D.J. Wolgemuth, Male sterility in mice lacking retinoic acid receptor alpha involves specific abnormalities in spermiogenesis, Differentiation 73 (4) (2005) 188–198.
[33] E.M. Snyder, C. Small, M.D. Griswold, Retinoic acid availability drives the asynchronous initiation of spermatogonial differentiation in the mouse, Biol. Reprod. 83 (5) (2010) 783–790.
[34] D.G. de Rooij, L.D. Russell, All you wanted to know about spermatogonia but were afraid to ask, J. Androl. 21 (6) (2000) 776–798.
[35] E. Evans, et al., Riding the spermatogenic wave: profiling gene expression within neonatal germ and sertoli cells during a synchronized initial wave of spermatogenesis in mice, Biol. Reprod. 90 (5) (2014) 108.
[36] P.M. Aponte, et al., Spermatogonial stem cells: characteristics and experimental possibilities, APMIS 113 (11–12) (2005) 727–742.
[37] K.S. Agrimson, et al., Characterizing the spermatogonial response to retinoic acid during the onset of spermatogenesis and following synchronization in the neonatal mouse testis, Biol. Reprod. 95 (4) (2016) 81.
[38] E.M. Snyder, et al., Exposure to retinoic acid in the neonatal but not adult mouse results in synchronous spermatogenesis, Biol. Reprod. 84 (5) (2011) 886–893.
[39] C. Zimmermann, et al., Research resource: the dynamic transcriptional profile of sertoli cells during the progression of spermatogenesis, Mol. Endocrinol. 29 (4) (2015) 627–642.
[40] S.R. Chen, Y.X. Liu, Regulation of spermatogonial stem cell self-renewal and spermatocyte meiosis by Sertoli cell signaling, Reproduction 149 (4) (2015) R159–R167.
[41] P.K. Nicholls, et al., Retinoic acid promotes Sertoli cell differentiation and antagonises activin-induced proliferation, Mol. Cell. Endocrinol. 377 (1–2) (2013) 33–43.
[42] S.B. Kurlandsky, et al., Plasma delivery of retinoic acid to tissues in the rat, J. Biol. Chem. 270 (30) (1995) 17850–17857.
[43] G. Livera, et al., Regulation and perturbation of testicular functions by vitamin a, Reproduction 124 (2) (2002) 173–180.
[44] T.J. Doyle, et al., Potential functions of retinoic acid receptor A in sertoli cells and germ cells during spermatogenesis, Ann. N. Y. Acad. Sci. 1120 (2007) 114–130.
[45] E.J. Jauregui, et al., Retinoic acid receptor signaling is necessary in steroidogenic cells for normal spermatogenesis and epididymal function, Development 145 (13) (2018).
[46] A. Gely-Pernot, et al., Spermatogonia differentiation requires retinoic acid receptor gamma, Endocrinology 153 (1) (2012) 438–449.
[47] I.C. Gaemers, et al., Differential expression pattern of retinoid X receptors in adult murine testicular cells implies varying roles for these receptors in spermatogenesis, Biol. Reprod. 58 (6) (1998) 1351–1356.
[48] A.M. van Pelt, et al., Changes in retinoic acid receptor messenger ribonucleic acid levels in the vitamin A-deficient rat testis after administration of retinoids, Endocrinology 131 (1) (1992) 344–40.
[49] N. Vernet, et al., Retinoid X receptor beta (RXRB) expression in Sertoli cells controls cholesterol homeostasis and spermiation, Reproduction 136 (5) (2008) 619–626.
[50] J. Zhang, et al., Neuregulins are essential for spermatogonial proliferation and meiotic initiation in neonatal mouse testis, Development 138 (15) (2011) 3159–3168.
[51] N.R. Peer, et al., Germ cell-specific retinoic acid receptor alpha functions in germ cell organization, meiotic integrity, and spermatogonia, Endocrinology 159 (9) (2018) 3403–3420.
[52] E.K. Velte, et al., Differential RA responsiveness directs formation of functionally-distinct spermatogonial populations at the initiation of spermatogenesis in the mouse, Development 146 (12) (2019 May 13), https://doi.org/10.1242/dev.173088 pii: dev173088.
[53] C. Niederberger, Re: oral administration of a retinoic acid receptor antagonist reversibly inhibits spermatogenesis in mice, J. Urol. 187 (4) (2012) 1509.
[54] S.S. Chung, X. Wang, D.J. Wolgemuth, Prolonged oral administration of a pan-retinoic acid receptor antagonist inhibits spermatogenesis in mice with a rapid recovery and changes in the expression of influx and efflux transporters, Endocrinology 157 (4) (2016) 1601–1612.
[55] J. Bowles, et al., Retinoid signaling determines germ cell fate in mice, Science 312 (5773) (2006) 596–600.
[56] J. Koubova, et al., Retinoic acid regulates sex-specific timing of meiotic initiation in mice, Proc. Natl. Acad. Sci. U. S. A. 103 (8) (2006) 2474–2479.
[57] J.M. Dufour, et al., Peroxisome proliferators disrupt retinoic acid receptor alpha signaling in the testis, Biol. Reprod. 68 (4) (2003) 1215–1224.
[58] Q. Zhou, et al., Expression of stimulated by retinoic acid gene 8 (Stra8) and maturation of murine gonocytes and spermatogonia induced by retinoic acid in vitro, Biol. Reprod. 78 (3) (2008) 537–545.
[59] A. Gely-Pernot, et al., Retinoic acid receptors control spermatogonia cell-fate and induce expression of the SALL4A transcription factor, PLoS Genet. 11 (10) (2015) e1005501.
[60] Y. Yang, et al., BMP4 cooperates with retinoic acid to induce the expression of differentiation markers in cultured mouse spermatogonia, Stem Cells Int. 2016 (2016) 9536192.
[61] A. Gely-Pernot, et al., Retinoic acid receptors control spermatogonia cell-fate and induce expression of the SALL4A transcription factor, PLoS Genet. 11 (10) (2015).
[62] S. Koli, A. Mukherjee, K.V.R. Reddy, Retinoic acid triggers c-kit gene expression in spermatogonial stem cells through an enhanceosome constituted between transcription factor binding sites for retinoic acid response element (RARE), spleen focus forming virus proviral integration oncogene (SPFI1) (PU.1) and E26 transformation-specific (ETS), Reprod. Fertil. Dev. 29 (3) (2017) 521–543.
[63] J.T. Busada, et al., Retinoic acid regulates kit translation during spermatogonial differentiation in the mouse, Dev. Biol. 397 (1) (2015) 140–149.
[64] L. Zhang, et al., C-kit expression profile and regulatory factors during spermatogonial stem cell differentiation, BMC Dev. Biol. 13 (2013) 38.
[65] M.C. Adolfi, et al., Retinoic acid and meiosis induction in adult versus embryonic gonads of medaka, Sci. Rep. 6 (2016) 34281.
[66] H.T. Ma, et al., Stimulated by retinoic acid gene 8 (Stra8) plays important roles in many stages of spermatogenesis, Asian J Androl 20 (5) (2018) 479–487.
[67] E.L. Anderson, et al., Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice, Proc. Natl. Acad. Sci. U. S. A. 105 (39) (2008) 14976–14980.
[68] F. Baudat, et al., Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11, Mol. Cell 6 (5) (2000) 989–998.
[69] K. Yoshida, et al., The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis, Mol. Cell 1 (5) (1998) 707–718.
[70] H. Xu, et al., Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis, Dev. Cell 8 (6) (2005) 949–961.
[71] L. Yuan, et al., The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility, Mol. Cell 5 (1) (2000) 73–83.
[72] A.M. Jequier, S.C. Holmes, Primary testicular disease presenting as azoospermia or oligozoospermia in an infertility clinic, Br. J. Urol. 71 (6) (1993) 731–735.
[73] J.K. Amory, et al., Isotretinoin administration improves sperm production in men with infertility from oligoasthenozoospermia: a pilot study, Andrology 5 (6) (2017) 1115–1123.
[74] I. Perrotta, et al., Expression and subcellular localization of retinoic acid receptor-alpha (RARalpha) in healthy and varicocele human spermatozoa: its possible regulatory role in capacitation and survival, Appl. Immunohistochem. Mol. Morphol. 23 (5) (2015) 374–381.
[75] T.F. Kolon, et al., Evaluation and treatment of cryptorchidism: AUA guideline, J. Urol. 192 (2) (2014) 337–345.
[76] S. Yang, et al., Generation of haploid spermatids with fertilization and development capacity from human spermatogonial stem cells of cryptorchid patients, Stem Cell Reports 3 (4) (2014) 663–675.
[77] K. Mandal, et al., Sertoli cell specific knockdown of RAR-related orphan receptor (ROR) alpha at puberty reduces sperm count in rats, Gene 641 (2018) 18–24.