Vitamin A and Male Reproduction

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Vitamin A is essential for various functions in the human body, including growth, epithelial differentiation, eyesight, immune system function, men's reproduction, and male infertility by enhancing sperm quality, bioenergetics, functioning, maintaining mitochondrial stability, and reducing oxidative damage. Males require dietary retinoid or vitamin A for spermatogenesis, as spermatogonial stem cells start the process through periodic RA after birth. Blood-testis barrier interactions and RA signals are crucial to the process of spermatogenesis.Spermatogenesis is a significant inducer of spermatogenesis in the mammalian testis. The undifferentiated spermatogonia are started by RA at the beginning of the 72-day spermatogenesis process in humans.

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Vitamin A and Male Reproduction

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Abstract:

Vitamin A is essential for various functions in the human body, including growth, epithelial differentiation, eyesight, immune system function, men's reproduction, and male infertility by enhancing sperm quality, bioenergetics, functioning, maintaining mitochondrial stability, and reducing oxidative damage. Males require dietary retinoid or vitamin A for spermatogenesis, as spermatogonial stem cells start the process through periodic RA after birth. Blood-testis barrier interactions and RA signals are crucial to the process of spermatogenesis. Spermatogenesis is a significant inducer of spermatogenesis in the mammalian testis. The undifferentiated spermatogonia are started by RA at the beginning of the 72-day spermatogenesis process in humans. The immune system, which is aided by vitamin A, mediates reactive oxygen species (ROS) activity and protects reproductive organs from oxidative stress. Beta-carotene (inactivated) and retinol (activated) are the two major forms of vitamin A that circulate in the body. Retinoic acid may contribute to male fertility through its impact on sperm quantity and shape. Vitamin A deficiency can stop spermatogenesis prematurely, but long-term chronic high vitamin A intake harms sperm viability, production, and morphology. Vitamin A measurement is crucial for understanding lipid peroxidation, which is a significant risk factor for human spermatozoa. Infertile patients may have higher levels of reactive oxygen species production, which could be a cause of idiopathic infertility. The body's defenses against oxidative damage rely heavily on lipid-soluble antioxidants like vitamin A, which includes beta-carotene. High levels of these vitamins have protective effects against disease by reducing lipid peroxidation. Techniques for measuring retinol include immunoassay technology, reverse phase open column chromatography, and monoclonal antibody-based immuno-enzymometric tests. High-performance liquid

chromatography (HPLC) is a quick and reliable method for estimating retinol levels simultaneously in serum and seminal fluid.

1. Introduction

The human body uses vitamin A for a variety of purposes, including growth, epithelial differentiation, eyesight, immune system function, and reproduction **[1].** Retinoic acid (RA), a vitamin A metabolite, is essential for the development of mammalian oocytes and sperm. When exposed to RA, the germ cells in the fetal ovary begin the process of meiosis, but the germ cells in the fetal testis are shielded from RA and do not do the same. Males require dietary retinoid or vitamin A for spermatogenesis since spermatogonial stem cells start the process through periodic RA after birth **[2,3].** Blood-testis barrier interactions and RA signals are recognized to be crucial to the process of spermatogenesis **[4].** The WHO established 1.4 ml for semen volume, 16 10^6/ml for semen concentration, 30% for progressive motility, 54% for viability, and 4% for sperm morphology as the lower limit values for each of the semen parameters. Spirogram anomalies have reportedly been found in measurements below these ranges, and this circumstance increases the chance of infertility **[5,6,7].** The process of spermatogenesis is significantly influenced by RA. The process by which spermatogonia transform into fully developed spermatozoa is known as spermatogenesis **[8].** In this procedure, retinoic acid-binding receptors' (RAR) signal transduction is essential. This process also entails several transcriptional processes that are controlled by many factors, including RAR, which regulates the transition from round spermatids to elongated spermatozoa and the start of the meiotic process from the spermatogonium to primary spermatocytes **[8].** Additionally, RA controls the expression of genes and hormones **[9].** The normal growth of the male adult reproductive system depends on RA. Through the preservation of germ cell proliferation and differentiation, RA controls the later stages of spermatogenesis after participating in Sertoli cell differentiation **[10].** By causing pachytene arrest in the first meiotic prophase, RA

further enhances the meiotic process **[11].** To develop into spermatozoa, the spermatogonium travels a great distance. At each stage of this procedure, RA causes immature germ cells to differentiate and change. RA is a derivative of vitamin A that is created in the body from other vitamins or from compounds that are consumed, such as retinal **[11].** RA is one of the most significant inducers of spermatogenesis in the mammalian testis **[12].** The undifferentiated spermatogonia are started by RA at the beginning of the 72-day spermatogenesis process in humans. It is crucial to remember that RA is nevertheless created in the fetal testis, specifically at the mesonephros during the embryonic period. Meiosis at the fetal stage can be stopped by RA **[12].** This suggests that when RA is active, it controls meiotic divisions during spermatogenesis. It guarantees that the spermatogenesis process proceeds without interruption, leading to the generation of haploid spermatids **[12].** The morphological alterations in the spermatogenic epithelium cause the generated haploid spermatid to develop into spermatozoa **[12].** The function of RA during the spermatogenic stages is shown in (**Figure 1)** below. Due to the effects of enzymatic inhibitors, particularly those from stages I to III, the concentration of RA is low at the beginning of the cycle. The germ and Sertoli cells are the sources of RA in this process **[12].** By raising RBP levels via a variety of channels and mechanisms, the RA plays a crucial part in controlling testicular function. For spermatozoa to grow and develop normally, RA is necessary. While spermatozoa solely use endogenous metabolite concentrations, RA signaling is necessary for spermatogonium and spermatocyte formation **[13].** The significance of these metabolites during spermatogenesis is shown by the necessity of retinoids in sperm formation and their capacity to restore male fertility when applied topically.

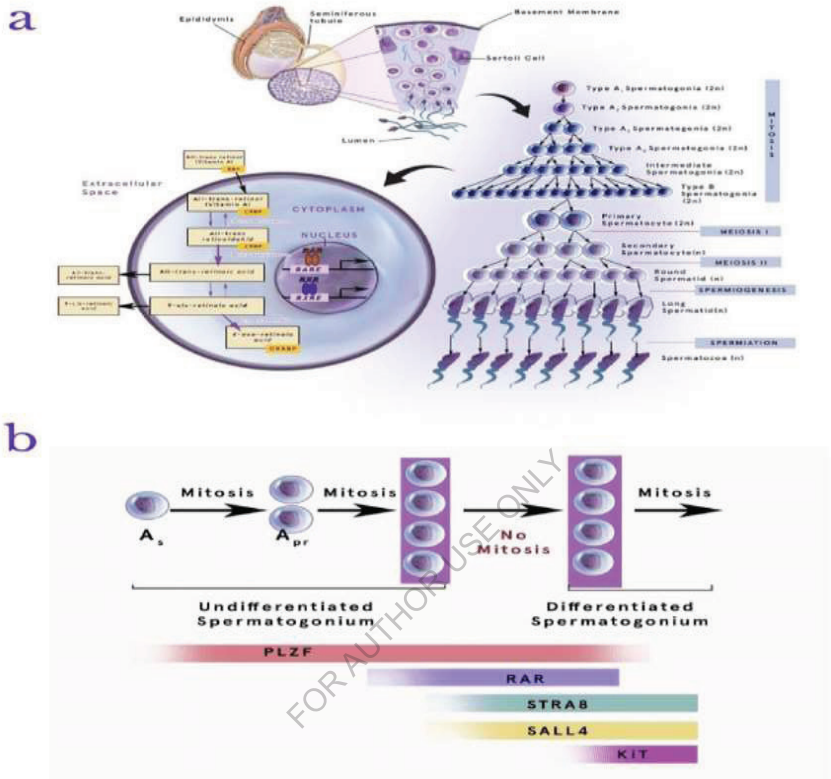


Figure 1. The steps of spermatogenesis and retinoic acid (RA) metabolism. In the spermatogenesis, RA is involved. As a result of metabolism, all-trans-retinol will enter the cytoplasm and be changed into 4-oxo-retinoic acid. This substance will be used by spermatogonia during the mitotic and meiotic stages of development to produce spermatozoa (a). The participation of RA in

spermatogenesis also involves several proteins, one of which is activated by the retinoic acid receptor alpha (RAR-), retinoic acid gene 8 (Stra8), promyelocytic leukemia zinc finger (Plzf), Spalt-like 4 (SALL4), and e-KIT. The conversion of the Apr spermatogonia into intermediate spermatogonia is mostly mediated by these proteins. The mitotic process may come to an end if these proteins do not engage with RA (b). RXR: retinoid X receptors, RXRE: retinoid X receptors elements, RAR-: retinoic acid receptor alpha, Stra8: stimulated by retinoic acid gene 8, Plzf: promyelocytic leukemia zinc finger, SALL4: Spalt-like 4, RARE: retinoic acid response elements.

2. Vitamin A

In terms of the health of both male and female reproduction, vitamin A is crucial. The immune system, which is aided by vitamin A, mediates reactive oxygen species (ROS) activity and safeguards the reproductive organs from oxidative stress **[14].** Beta-carotene (inactivated) and retinol (activated) are the two major forms of vitamin A that circulate in the body. Through its impact on the control of sperm quantity and shape, retinoic acid, another vitamin A metabolite, may contribute to male fertility **[14–16].** Throughout childhood and the early stages of development, retinoic acid stimulates spermatogenesis **[17].** Men with normozoospermia have been found to have higher serum retinol levels than men with oligospermia, asthenozoospermia, and azoospermia **[14].** Retinoic acid prevents meiosis I/II and post-meiotic spermatid development, which takes place behind the immunological blood-testis barrier (BTB), which compromises the BTB. The seminiferous epithelium of the epididymis, prostate, and seminal vesicle is damaged by vitamin A deficiency, which stops spermatogenesis **[17].** Even though vitamin A deficiency causes spermatogenesis to end prematurely, the long-term chronic high vitamin A intake harms sperm viability, production, and morphology **[18].** Circulating beta-carotene is converted to retinol by the beta-carotene mono-oxygenase 1 (BCMO1) enzyme, which is encoded by the BCMO1 gene **(Figure 2).** 70% of people carry the BCMO1 (rs11645428) gene's GG variation, which is linked to an ineffective conversion of beta-carotene to retinol **[19].** Retinoic acid induces the expression of STRA8 and REC8, two genes involved in human spermatogenesis **[20].** The tight junction proteins that strengthen the BTB are encoded by genes that contain unidentified sequence variations.

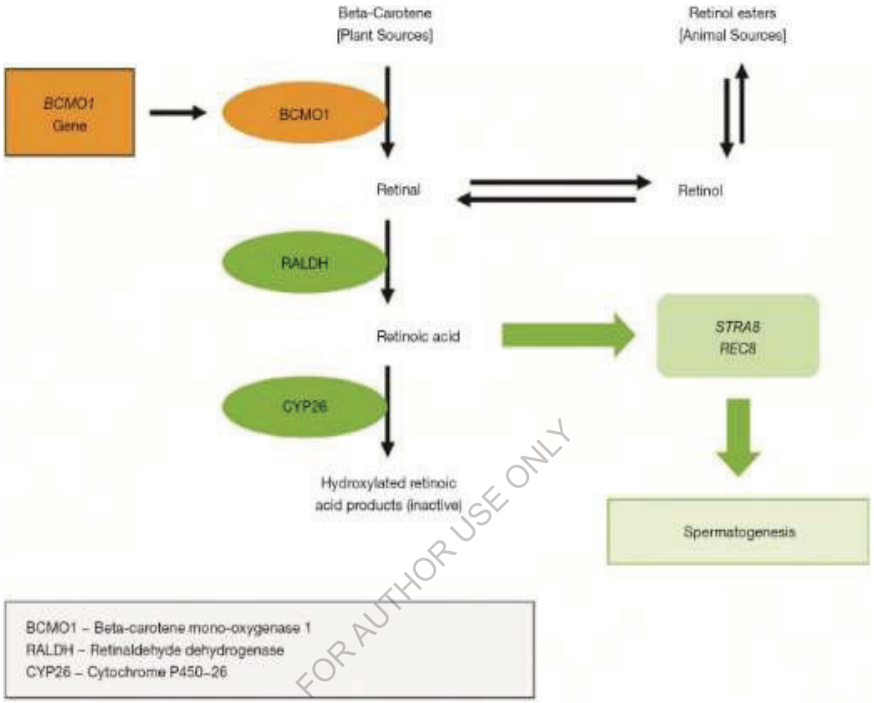


Figure 2. The metabolic process of vitamin A. Circulating beta-carotene is transformed to retinal (aldehyde) by beta-carotene mono-oxygenase 1 (BCMO1), and the aldehyde form is then changed into retinoic acid. STRA8 and REC8 expression is induced by retinoic acid, which supports spermatogenesis.

3. Historical perspective

For decades, it has been known that proper spermatogenesis requires dietary retinol or vitamin A **[21–24].** The mechanisms underlying the precise requirements for retinol and its active metabolite all-trans-retinoic acid (ATRA) in controlling spermatogenesis have yet to be fully understood, even though significant insight has been gained into the molecular basis for this requirement, particularly from targeted mutagenesis of genes important at various levels of retinoid metabolism, transport, and receptor activity. The alterations in spermatogenesis caused by the mutation of the RAR receptor gene were recognized to be comparable to those seen in the vitamin A-deficient (VAD) testis **[25],** Furthermore, it is currently unclear whether the induction of VAD in the animal after spermatogenesis has been established would phenocopy the defects seen in testes deprived of ATRA signaling from conception (RAR-deficient mice). On the other hand, it's not quite apparent if all of the consequences of VAD would appear in animals lacking just one retinoic acid receptor, as the RAR-deficient. Numerous genes with retinoic acid response elements (RAREs) or genes with ATRA-induced up- or down-regulation have been are targets of RAR, but it is unknown which of these genes might be crucial for spermatogenesis, a crucial physiological target of vitamin A function **[26].**

4. Vitamin A after ingestion

All vitamin A required by humans must be obtained from the diet as provitamin A carotenoids (such as -carotene from plants) or preformed vitamin A (primarily retinyl esters and retinol from animal sources), which are then transformed in the body to retinal and ATRA **[27-29]**. In the small intestine, one or more retinyl ester hydrolases (REHs) hydrolyze dietary retinyl esters (REs) to retinol. Retinal can then be converted to retinol by cleaving provitamin A carotenoids. Cellular retinol-binding protein type II (CRBP-II) binds retinol, which is then esterified to REs by the enzyme lecithin: retinol acyltransferase (LRAT). Then, REs are bundled into chylomicrons with other dietary lipids and secreted into the lymphatic system. Hepatocytes in the liver internalize the chylomicron REs. Retinol is coupled to cellular retinol-binding protein, type I (CRBP-I) within hepatocytes and hepatic stellate cells. It has been suggested that freshly produced serum retinol-binding protein (RBP) is transported retinol through CRBP-I. Then, to supply the tissue with vitamin A, the RBP-retinol combination is released into the bloodstream. Retinol can also be esterified to RE, which is the main form of storage, by LRAT in the hepatocytes **[30,31].** Retinol is either transformed into active metabolites like retinals and retinoic acids within cells or esterified for storage **[28,32].** Retinal-to-retinal conversion is reversible, whereas retinal-to-ATRA conversion is irreversible. Animals kept on a VAD diet and supplemented with ATRA are almost free of all VAD symptoms, except blindness and sterility in the males **[22].** It is probably because of the blood-testis or Sertoli cell barrier that dietary retinol, not ATRA, is necessary for the maintenance of spermatogenesis. Less than 1% of ATRA in the rat testis has been found to come from the plasma pool, suggesting that serum-bound retinol must be the source of testicular ATRA **[33]**.

5. Role of retinoid-specific binding proteins

Retinoids need to be coupled to proteins in an aqueous environment intoetain their solubility **[48].** There are many known retinoid-specific binding proteins, some of which are intracellular and others external. RBP, transthyretin (TTR), and interphotoreceptor retinoid-binding protein (IRBP) are extracellular, whereas CRBP-I and CRBP-II and cellular retinoic acid binding protein, types I and II (CRABP-I and CRABP-II) are exclusively intracellular. According to a theory put out by researchers, CRABPs may serve as "buffers" to regulate spatiotemporally the precise amount of "free" intracellular retinoic acid that is accessible to bind to nuclear receptors **[34].** In circulation, RBP is the only specific transport protein for retinol, and it was suggested that one of its physiological functions is to deliver retinol to tissues. The glomerular filtration of RBP has been demonstrated to be prevented by TTR, an abundant serum protein made up of four 14-kDa monomers that are found in 1:1 interaction with RBP **[35].** The peritubular myoid cells that encircle the seminiferous tubules have been demonstrated to have a role in the transfer of serum retinol to Sertoli cells via binding RBP to TTR. According to in vitro research, retinol is likely transported to the peritubular cells in a combination with both RBP and TTR before being re-secreted linked to RBP produced by the peritubular cells themselves **[36].** Rat Sertoli cells were also demonstrated to produce and secrete RBP protein in vitro **[36].** The notion that serum retinol is chaperoned by RBP and TTR first to the peritubular cells, and then to the Sertoli cells, was based on the fact that tritiated retinol is absorbed from retinol-RBP or retinol-RBP-TTR complexes by cultured Sertoli cells **[23,37].** Although it is believed that Sertoli cells bound by RBP-TTR absorb retinol, gene targeting to eliminate either Rbp or Ttr has not shown any effects on spermatogenesis **[38,39].** Rajguru and colleagues (1982) **[40]** used autoradiography to show that labeled retinol is largely localized to three cellular locations in the adult rat testis:

spermatids in connection with Golgi saccules, lipid droplets of the Sertoli cells, and macrophages of the interstitial tissue. Retinol or its metabolites cannot diffuse into the adluminal compartment, which houses the spermatocytes and spermatids, because of the blood-testis barrier, which is mediated by junctions in the Sertoli cells meaning, neither retinol-RBP nor ATRA can diffuse directly to these cells. Only Sertoli cells and spermatogenic cells outside the barrier, or the basal compartment, can be reached by retinol complexed with RBP. Therefore, it is still unknown how the tagged retinol got to the spermatids. Cells can more easily absorb retinol from circulating retinol-RBP complexes when CRBP is present. Exclusively CRBP-I, whose expression is exclusively seen in Sertoli and peritubular myoid cells, hasisesent in the testis among members of this family **[23].** Because of this, only Sertoli cells in the testis can absorb retinol from bloodstream retinothe l-RBP complexes. In rat Sertoli cells, CRBP-I expresses differently depending on the stage, peaking between stages XII and XIII and peaking between stages VI and VIII. CRBP-III is primarily found in the liver and kidney **[41],** As opposed to CRBP-IV, which is mostly expressed in the kidney, heart, and transverse colon, this indicates that various intracellular mediators of retinol metabolism work in various organs **[42].** The male and female reproductive tracts, including the testis, were shown to have CRABP-I in exceptionally high concentrations **[43].** ATRA is known to be rapidly converted inside cells into several retinoid metabolites, some of which are inactive **[44].** As a result, retinoic acid must likely be produced right before it acts and close to where it acts, most likely inside the target cells. This theory is supported by the discovery of retinoic acid synthesis and breakdown enzymes in the testis. It has been demonstrated that CRABPs function to transport ATRA to the nucleus **[45].** Recent research on the molecular basis of CRABP function has revealed that in COS-7 and MCF-7 cells, the direct channeling of ATRA between CRABP-II (and not CRABP-I) and RAR

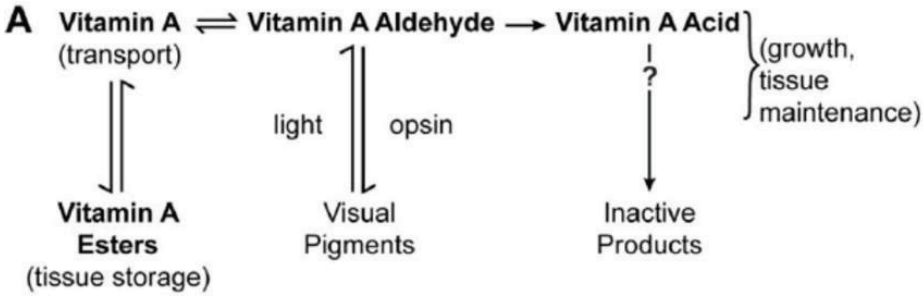
is necessary for ATRA to travel to the RAR receptor **[46,47].** In the testis, CRABP-I was never shown in Sertoli cells and was only ever found in the cytoplasm of embryonic gonocytes and spermatogonia of the post-natal and adult testis, including intermediate and B-type spermatogonia **[48-50].** The fact that CRABP-I is only found in the cytoplasm of gonocytes and spermatogonia suggests that it may play a role in securing ATRA in the cytoplasm of these mitotically dividing germ cells, which are not subject to the Sertoli cells-mediated blood-testis barrier. This would prevent ligand-dependent activation of the nuclear retinoic acid receptors **[50]** CRABP-I may not be required in cells past the barrier, which effectively sequesters their exposure to ATRA, based on the lack of expression in more advanced spermatogenic cells. Using a well-characterized CRABP-II antibody, CRABP-II expression in the embryonic testis was conclusively localized to Leydig and Sertoli cells in contrast to CRABP-I **[43,40].** Immunostaining allowed for the early detection of CRABP-II protein in rat fetuses as early as day 16 **[50].** Relatively high quantities of CRABP-II mRNA were discovered on postnatal day (pnd) 4 before rapidly declining to undetectable levels on pnd 20. The timing of Sertoli cell proliferation in the rat testis, which is maximum at day 20 of gestation and declines until pnd 21, coincides almost perfectly with the developmental pattern of expression of CRABP-II in fetal and prepubertal Sertoli cells **[51–53].** Zheng and colleagues were inspired by these similarities in expression patterns **[50].** To suggest that CRABP-II participates in the autocrine or paracrine regulation of Sertoli cell proliferation that is dependent on retinoic acid.

6. The transport and metabolism of vitamin A

The transport, metabolism, and degradation of retinoids, which are chemically related to vitamin A, as well as the activation of its nuclear receptors, are the mechanisms by which vitamin A exerts its effects in the testis **[54].** Since serum cannot effectively transport RA, target tissues are typically where dietary vitamin A (retinol) is converted to RA. To strictly control the synthesis and breakdown of RA, retinol is stored in the liver and delivered to target tissues **[55]** that express the necessary intracellular binding proteins and retinoid metabolism enzymes **[56].** The expression patterns of a few retinoid metabolism enzymes were studied by Chung and Wolgemuth **[57]** and Vernet et al. **[58]** to determine how their activity may influence spermatogenesis and fertility. In cells, RA binds to two types of intracellular receptors known as retinoid X receptors (RXRs), which bind 9-cis RA, and RA receptors (RARs), which bind all-trans and 9-cis RA **[54].** By attaching to particular components in the promoter regions of genes under the control of vitamin A, these receptors, which exist as homo- and heterodimers, influence gene expression. There is an agreement that RA exerts its effects predominantly through the activity of RARα in Sertoli cells and through the action of RARγ in early germ cells, even though many of the isoforms of RAR and RXR are present in distinct testis cell types at different developmental stages. Males with the mutant Rara allele are infertile, although transplantation studies showed that germline stem cells containing these alleles could colonize a testis that was lacking in germ cells and proceed through spermatogenesis properly **[59].** This implies that RARα expression in Sertoli cells is essential for healthy spermatogenesis. RARα signaling affects Sertoli cells, although its exact mode of action is still unknown. It is obviControllingssion of RA receptors is crucial for mammalian spermatogenesis and fertility because the deletion of Rxrb in male mice fails spermatid release and

testis degeneration **[60].** In many aspects, retinol uptake and the preservation of massive pools of this retinoid appear to be identical processes carried out by the liver and Sertoli cells **[54].** Retinoids may also be stored in spermatids, testicles, and epididymal sperm, according to some research **[61].** It has been suggested that Sertoli cells "distribute" RA to germ cells because they are the primary location of RA synthesis in the testis **[54].** Additionally, retinol might be delivered to germ cells through Sertoli cells, or spermatogonia could directly obtain retinol and RA from serum through the vascular system **[54,58].** RA appears to be responsible for the differentiation of undifferentiated spermatogonia, the beginning of meiosis, and possibly the beginning of the cycle of the seminiferous epithelium. There is evidence to suggest that the delivery of RA to germ cells is tightly regulated and that different germ cells can respond to RA in different ways. All-trans retinol, often known as vitamin A, can be found in food as carotenoids with vitamin A activity from plant sources or as retinyl esters from animal sources. There are two main effects of retinol: (1) All-trans retinaldehyde is produced through oxidative metabolism, which is then converted into RA (2) Tissue storage and esterification. Lecithin retinol acyltransferase (LRAT) is an enzyme that converts the majority of retinol into retinyl esters **[62].** The first and rate-limiting step in the synthesis of RA from retinol is carried out by cytosolic alcohol dehydrogenases (ADH) and microsomal retinol dehydrogenases (RDH), which lead to the generation of all-trans retinaldehyde **[63].** All-trans retinaldehyde is irreversibly oxidized to all-trans retinoic acid by several aldehyde dehydrogenases (RALDH), also known as RALDH 1, 2, and 3 **[64,65].** It has also been documented that CYP1B1 can produce RA from retinol without the aid of RALDH **[66].** Cytochrome P450 enzymes of the CYP26 family (A1, B1, and C1) convert RA at the C4 and C18 positions to oxidative metabolites such as 4-hydroxy-RA, 18-hydroxy-RA, and 4-oxo-RA **[67].** Lipophilic substances like vitamin A and its metabolites are typically

detected along with serum and cellular binding proteins **[68].** The majority of retinol in circulation is carried by retinol-binding protein (RBP or RBP4), and STRA6 interacts with RBP to facilitate efficient retinol uptake by several cells **[69].** Additionally, cellular proteins that bind to retinol (CRBP I, II, and III) and RA (CRABP I and II) have been investigated in null mutant mice; some of these proteins are not necessary, while others play important roles when animals are fed a diet low in vitamin A **[62].** The nuclear retinoic acid receptor (RAR) proteins bind to RA. RAR protein is divided into three main subtypes and additional isoforms produced by alternative promoter use and alternative splicing **[70].** The nuclear RARs function as tissue- and cell-specific ligand-activated transcription factors to control gene transcription **[72].** The greatest affinity endogenous ligand for the RAR is the all-trans isomer of RA **[73].** The retinoid (rexinoid)-X receptors (RXR), which belong to a different protein family, heterodimerize with RAR to give DNA high-affinity binding. Retinoic acid response element (RARE) refers to the DNA that the RAR/RXR heterodimer binds to. The two direct repeats of PuG(G/T)TCA that make up the consensus RARE are often spaced apart by five bases **[74**,**75].** When occupied by the RA/RAR/RXR complex, RAREs act as enhancer elements and promote chromatin opening and alterations in the transcriptional activity of RA target genes **[76,77].** Cells or tissues exposed to RA modify a vast number of genes, although only a small portion are primary (direct) targets via RARE-mediated transcription, with the balance being downstream targets **[78,79]. (Figure 3)** illustrates a schematic of vitamin A metabolism and the biological mechanism of RA activity. The term "retinoid" refers to substances that share a structural similarity with retinol and is used to describe vitamin A and its metabolites.



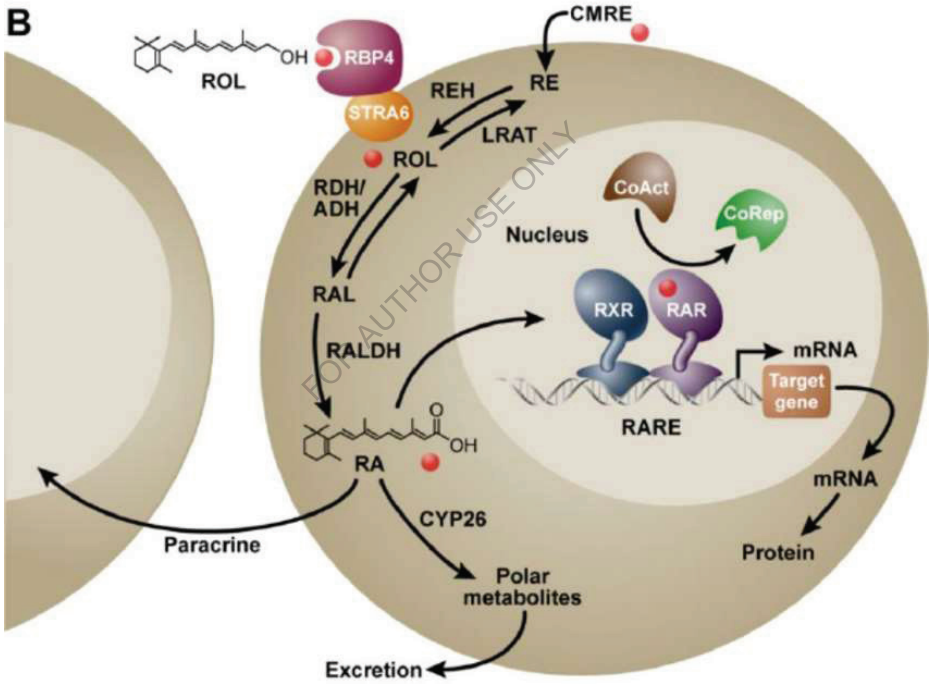


Figure 3. All-trans retinoic acid (RA) is produced by the breakdown of vitamin A (retinol) and RA's mode of action. (A) Dowling and Wald's 1960 **[114]** metabolic scheme; (B) a mechanism from around 2011. Retinol, or vitamin A, is transported through the bloodstream attached to transthyretin and the plasma retinol-binding protein 4 (RBP4). In some cells, RBP4 interacts with membrane receptor STRA6 to promote retinol absorption. The chylomicron remnant (CMRE), which contains vitamin A, can also provide the cell with vitamin A. The omission of cellular retinol and RA-binding proteins was done so that the analysis would be simpler. Retinol is either preserved after being esterified by the enzyme lecithin: retinol acyltransferase (LRAT) or it is oxidized irreversibly to retinol by the enzyme retinaldehyde dehydrogenase (RALDH 1, 2, or 3). The retinoic acid response element (RARE), a particular DNA sequence, serves as the binding site for the RAR/RXR complex in the nucleus. The release of the corepressor complex (CoRep) and association with coactivator proteins (CoAct) that result from the binding of RA to the RAR influence the transcription of downstream target genes, which ultimately affects cellular function. The cytochrome P450 (CYP) 26 family also further oxidizes RA to produce more polar metabolites. The lipophilic molecule RA can function either autocrine—within the cell in which it is produced—or paracrine—within neighboring cells.

Abbreviations: Alcohol dehydrogenase (ADH), retinol dehydrogenase (RDH), retinyl ester hydrolase (REH), and retinyl ester (RE).

7. Vitamin A and Reproduction

For males to reproduce, they need vitamin A. Early research conducted in the labs of Wolbach, Howe, and Mason showed that spermatogenesis stops and the epithelia of the epididymis, prostate, and seminal vesicle are replaced in vitamin A deprivation **[80].** When vitamin A is added, spermatogenesis can be restarted by synchronizing the A to A1 spermatogonial differentiation **[81–83].** (**Figure 4)** depicts the stop in adult spermatogenesis brought on by a vitamin A shortage. Recent studies confirm the need for the vitamin A metabolite RA for adult male spermatogonial differentiation (transition to A1) and meiosis entry **[84-86].** As evidence that RA is the active form of vitamin A in male reproduction, Van Pelt and de Rooij found in 1991 that a large dose of RA (5 mg) given by injection twice a week, along with an RA-containing diet, supported the development of spermatocytes and their subsequent development into spermatids in VAD rats **[87].** The seminiferous tubule may be surrounded by a peritubular myoid cell-mediated catabolic barrier that prevents RA from the general circulation from reaching cells inside the tubule, which would explain why such high dosages of exogenous RA were required **[88,89].** The Sertoli cell is thought to produce RA in the normal tubule through the action of Raldh1 **[88,90,91],** and potentially Raldh2 **[89].** Along with early-stage spermatids, Raldh2 is also present in late pachytene and diplotene spermatocytes. Males who lack RARγ are infertile and their seminal vesicles and prostate glands have undergone squamous metaplasia **[92].** Nuclear RARα is required for spermiation, as evidenced by the fact that RAR null mutants are sterile and have reduced spermatozoa **[93].** RARα is predominantly expressed in Sertoli cells, Rarβ in spermatids, and A spermatogonia **[88].** For the spermatogonia to differentiate throughout prepuberty, RARγ expression is required **[94].** However, since deletion of all RARs in the Sertoli cell does not result in the arrest of

spermatogonia differentiation in the adult mouse, RAR signaling in the Sertoli cell cannot explain the VAD-induced pause in spermatogonia differentiation. Research on the cell types in which RA and its receptors assist spermatogenesis is also ongoing **[84,88,94].**

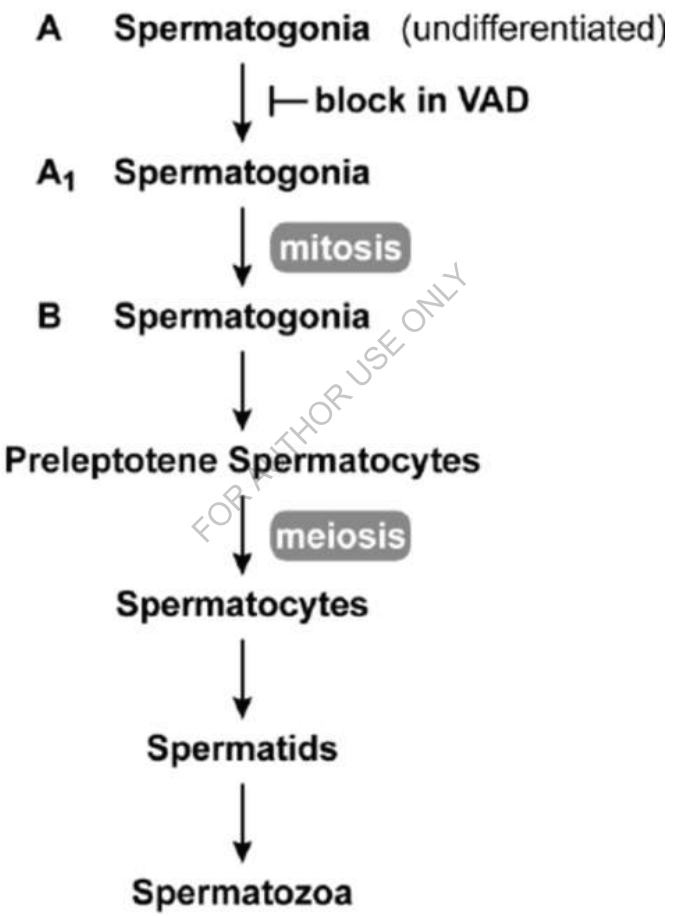


Figure 4. Adult spermatogenesis. From puberty to adulthood, spermatogenesis occurs in the seminiferous epithelium of the testis tubules. At the base of the seminiferous epithelium, undifferentiated (A-type) spermatogonia divide mitotically until they reach the differentiation pathway to become A1 spermatogonia. A1 spermatogonia divide into A1-A4 spermatogonia and then B spermatogonia. Preleptotene (primary) spermatocytes produced by dividing B spermatogonia go away from the base of the seminiferous tubule to perform meiosis. Secondary spermatocytes are produced during the first meiotic division, while spermatids (haploid cells) start to differentiate into spermatozoa during the second meiotic division. The transition from A to A1 spermatogonia is prevented in vitamin A deficiency **[81-83].**

8. Vitamin A and sperm DNA damage

Sperm DNA damage and sperm membrane lipid peroxidation are caused by oxidative stress in the reproductive system, which may be a significant factor in the pathophysiology of male infertility, particularly in idiopathic instances. Carotenoids are an example of an antioxidant that protects against free radical damage. Young couples are more likely than older couples to experience infertility, and more than 50% of instances are caused by male factors. Idiopathic conditions account for about 25% of male infertility causes **[95].** Reactive oxygen species (ROS) are released by leukocytes and abnormal sperm **[96].** Oxidative stress is a factor in male infertility and is brought on by the sperm's increased production of ROS and decreased capacity to scavenge them **[97].** In seminal plasma, a group of antioxidants with scavenging abilities keep ROS levels stable. Superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase are just a few of the antioxidant enzymes found in seminal plasma. Other non-enzyme antioxidants include ascorbate, alpha-tocopherol, urate, coenzyme Q10, albumin, N-acetyl-L-cysteine, pyruvate, taurine, and hypurate polyunsaturated fatty acids (PUFA) in the sperm membrane are attacked by ROS, which causes a buildup of lipid peroxidation products as well as the degradation and loss of PUFA. Sperm are less mobile, fluid, functional, and fertile as a result of this process **[98,99].** Keeping sperm alive for the transmission of genetic information and embryonic development, DNA integrity is crucial **[100].** DNA fragmentation, protein cross-reaction, and decreased oocyte fertility are all effects of sperm DNA damage. A reduction in the quality of semen can arise from genetic alterations brought on by ROS, including point mutations and polymorphisms **[101].** Mutagens include ROS, UV, gamma, X-rays, and alkali elements that alter and modify DNA **[100,102].** Alpha- and beta-carotene, beta-kryptogezantyne, lutein, and lycopene, which have

strong double bonds and exhibit the highest antioxidant activity, make up over 90% of dietary carotenoids. One of these, lycopene, has potent antioxidant properties, including the ability to scavenge free radicals and singlet oxygen **[103].** According to epidemiological research, a high tissue absorption of dietary carotenoids is favorably connected with carotenoid concentrations and negatively correlated with a high risk of developing chronic diseases **[103-105].** Lycopene and beta-carotene have been shown to have an inverse association with the incidence of cardiovascular disease and several malignancies **[103,106,107].** In contrast to other carotenoids, heat increases the absorption of lycopene by changing its Trans to cis isomer; the cis isomer is also more soluble in bile micelles and is hence more absorbable **[108].**

9. Vitamin A and spermatogenesis

Adult mammals have highly coordinated spermatogenesis, which aims to produce sperm continuously. Vertebrate testes are organized into recurrent cellular connections along the tubule that change over time and space. The spermatogenic wave and the cycle of the seminiferous epithelium are named for these variations across time and space, respectively.

9.1. Organization of spermatogenesis in time and space

Most male mammals' seminiferous epitheliums appear to be very well organized. At various phases, clearly defined groupings of germ cells may emerge sequentially along the tubule or at a single location on the tubule **[109].** These morphologically diverse clusters of germ cells referred to as stages (also known as cell associations), can be found in histological testis tubule. For instance, the mouse has 12 different developmental stages, and each stage is represented by a particular group of germ cells being present at the same moment at one location on the tubule **[110].** In a person, there are six such stages, each tubule that can be seen in a cross-section of an adult mouse testis can be categorized into one of several stages based on the germ cells that are found there. However, a longitudinal analysis of a tubule reveals that these stages are organized so that the associated germ cells naturally advance to the following stage of their development. The spermatogenic wave describes how germ cells move during each stage along a tubule. The cycle of the seminiferous epithelium would be visible if we were able to track the differentiation of germ cells over time at a single location on the tubule. This cycle would consist of a defined group of cell associations first appearing, followed by several others, and then the original group of associations reappearing. The number of stages in every given mammal is random (i.e., does not refer to germ cell development) and unique to the species. The technique for describing spermatogenesis that is most commonly used uses these numbered stage

schemes. There is a continuum of germ cell development. The somatic cells do not influence the timing of the progression of germ cell development, which is intrinsic to the germ cells of a particular species. This notion was made based on experiments in which mouse testes were given rat germ cells. The recipient mouse germ cells did not mature at the same time as the donor rat germ cells, and the cellular relationships that followed were those of the donor rat germ cells **[111].** Every 8.6 days, undifferentiated A spermatogonia make the switch into the differentiation pathway (i.e., transform into A1 spermatogonia) and subsequently enter meiosis at a single site along a mouse seminiferous tubule. The release of spermatozoa into the tubule lumen at that specific location on the tubule also occurs at the same time as this change. As a result, at any given place along a tubule, spermatogonia will develop at the tubule's base and release spermatozoa into the lumen every 8.6 days in mice (16 days in humans). Both of these processes take place in stage VIII in the mouse. Spermatozoa are released at the start of stage III in humans, whereas A-pale spermatogonia differentiation appears to occur during stages II–VI **[110].** However, the mouse is a great model for the study of mammalian spermatogenesis because it has cell types that are similar to those of humans and because both species have a cycle of germ-cell growth. makes it abundantly evident that the cycle of the seminiferous epithelium is produced by the temporally controlled entry of spermatogonia into the differentiation pathway. Spermatozoa are ultimately released at any given place along the tubule every 8.6 days as a result of the mouse cycle. The stages of the cycle are visible as one moves along the tubule in space; that is, adjacent stages are visible close to one another. The spermatogenic wave is simply a change in cellular connections that occurs in space rather than time **[112,113].** The temporally controlled entry of germ cells into the differentiation pathway can be phased along the tubule to produce the wave. When this happens along the full length of the tubule, the

wave's overall effects are the asynchronous phased release of spermatozoa at stage VIII of the cycle and the ongoing creation of spermatozoa.

9.2. Vitamin A deficiency in testis

The investigation of the effects of spermatogenesis upon vitamin A deficiency from food has been made possible by the revelation that ATRA could treat the majority of VAD symptoms, except abnormalities in eyesight and male fertility **[114,115].** Many studies have been done on the alterations that take place in the testis of the VAD rat **[116]** For instance, analysis of the timing and kinetics of spermatogenic cell loss in VAD-affected rat testes showed that starting on day 2, all stages of spermatids abruptly dropped after the initial loss of body weight (also known as the growth retardation phase of VAD). By day 10, spermatids had vanished from the tubules, whereas the number of primary spermatocytes drastically fell from days 5 to 12. Additionally, Sertoli cell-spermatid connection has been disrupted **[119],** spermiation has been delayed **[117,118],** and spermatid degeneration has also been noticed **[120,121].** The rapid disappearance of spermatids and spermatocytes from the tubules at the beginning of the growth retardation phase of VAD indicates that the mechanisms in charge of spermiogenesis, spermiation completion, and spermatocyte differentiation are very sensitive to changes in the status of vitamin A. The decline in spermatogonia was reported to be steady and relatively low during the time after the commencement of growth retardation **[117,118].** However, only around 25% of the spermatogonia (A and B type) remained by days 16 to 20, indicating a decline in the spermatogonial population. This implied that vitamin A was also necessary for the maintenance of the spermatogonial population. It is crucial to think about whether the effects of VAD originate from disruptions in Sertoli cell-germ cell interactions or directly affect the germ cells. The adluminal compartment of the tubules, where the spermatocytes and spermatids are located, has a unique milieu that is affected by

any malfunction of the specific junctions between Sertoli cells and germ cells or between Sertoli cells. Therefore, it is possible that the degeneration of these spermatogenic cells results from alteration of the milieu in the adluminal compartment brought on by inappropriate Sertoli cell function. When Ismail and Morales **[122]** examined the impact of VAD on tight junction development, they discovered that even during the severe regression that occurred three to four weeks following the onset of growth retardation, the tight junctions were still there. Even though Huang and colleagues **[119]** revealed that inter Sertoli cell tight connections were disrupted in rats on a VAD diet as early as 10 days after the growth retardation phase, problems in germ cells were noticed earlier. This shows that the degeneration of germ cells observed in VAD rats was a direct result of vitamin A deficiency rather than the collapse of the inter-Sertoli cell tight connections. Recent studies note that lanthanum nitrate deeply infiltrated the seminiferous cords in VAD rat testis (3–9 weeks after the onset of growth retardation), supporting the effect of VAD on Sertoli cell tight connections **[121].** from prolonged VAD (7-9 weeks from the onset of growth retardation), vitamin A replenishment may be able to reestablish the Sertoli cell barrier. Intercellular tracer continued to freely enter the inter-Sertoli gaps surrounding not only preleptotene spermatocytes but also zygotene and pachytene spermatocytes in the testes of animals in which vitamin A was restored and spermatogenesis was resumed **[123].** Thus, zygotene and pachytene spermatocytes were developing without a strict junctional barrier made by Sertoli cells, while some of these cells showed signs of death. Collectively, the adult VAD rat testis has at least four significant spermatogenesis abnormalities. They include spermatid degeneration, a breakdown of inter-Sertoli cell tight connections, failure of the creation of A2 spermatogonia from A1 spermatogonia at the outset of VAD, and a delay in the onset and aberrant advancement of meiotic prophase.

10. Vitamin A Measurement

Since lipid peroxidation is a significant danger factor for human spermatozoa, it is known that the addition of human spermatozoa to the incubation medium will inhibit sperm motility **[124,125].** According to Kessopoulou et al. **[126]** and Rajasekharan et al. **[124],** one of the main reasons for abnormal sperm function is peroxidative damage brought on by reactive oxygen species (ROS). According to research by Aitken and Clarkson **[127],** certain infertile patients have greater amounts of reactive oxygen species production, which suggests that this formation could be one of the causes of idiopathic infertility. The body's defenses against oxidative damage depend heavily on lipid-soluble antioxidants like vitamin A **[128].** In addition to retinol, vitamin A also includes beta carotene, a precursor to vitamin A that has one-sixth the vitamin activity of retinol **[129]** High levels of these vitamins also have protective effects against disease in general by reducing lipid peroxidation. The amount of retinol has been determined using a variety of techniques. They include immunoassay technology **[131],** reverse phase open column chromatography **[130],** and monoclonal antibody-based immuno-enzymometric tests of retinol-binding protein. **[132].** High-performance liquid chromatography (HPLC) has been utilized in recent years to quantitatively measure metabolites crucial to nutrition. **[133].**

Older methods, such as the production of chromophores or fluorophores without first separating them or the use of traditional microbiological assay techniques, are still employed in laboratories that lack powerful separation techniques like high-performance liquid chromatography (HPLC) or particular immunoassays **[133-139].**

The best specimen type for analysis should be chosen (urine, serum, plasma, semen, whole blood, washed red cells, white cells), along with the appropriate anticoagulant if necessary. In some situations, urine may provide more important

information than blood, but it is still necessary to collect it at a specified interval (such as 24 hours). Vitamin fluctuations in serum or plasma occur more quickly than in red blood cell fluctuations. Red cell concentrations may therefore better reflect body storage than serum or plasma concentrations, which may be more susceptible to changes in recent ingestion. Weight per volume or SI (molar) measurements can be used to express vitamin concentrations in biological fluids. Although the former is still used for foods and diets.

10.1 Vitamins A in serum or plasma

Retinol-binding protein and transthyretin, or thyroxine-binding pre-albumin, two hepatically produced proteins, form a 1:1:1 complex with vitamin A, which is primarily found in the plasma as the alcohol (retinol). During a period of deficit, the amount in circulation stays nearly constant as the body stores diminish until the liver stores are unable to support this typical circulating level in the plasma. Therefore, plasma vitamin A is only a reliable indicator of status in those with extremely low bodily storage. The chylomicrons temporarily store recently absorbed vitamin A as retinyl esters, and the majority of intracellular storage of vitamin A in tissues is present as esters with long-chain fatty acids. Retinaldehyde, the active center of the retina's visual pigment, and retinoic acid, an intracellular hormone with nuclear signaling activity, are examples of the functional forms found in the tissue. Vitamin A toxicity, whether acute or chronic and teratogenic consequences in developing fetuses are all risks associated with excessive dietary intakes of the vitamin. During severe vitamin A overload, plasma retinyl ester levels are increased, and clearance may be delayed. Thus, high readings may be a sign of toxicity concern. With the help of the B-carotene l5,15'-dioxygenase enzyme, some dietary carotenoids, particularly B-carotenes, can be converted to vitamin A in animals. This enzyme is located in the intestinal mucosa and other places. Indicators of fruit and vegetable intake may be found in other carotenoids,

such as lutein, zeaxanthin, and lycopene, which do not go through this conversion process. The majority of carotenoids can be ingested whole and are then transported throughout the body via the lipoproteins and chylomicrons of plasma.

10.2 Methods

Modern HPLC-based techniques may detect this set of vitamins A concurrently in a single aliquot of a biological sample, hence their measurement is now frequently combined into a single experiment. Older techniques before the invention of HPLC, methods that did not use separation but instead depended on particular chemical reactivity or the distinctive physicochemical features of the vitamins were employed. These included direct fluorimetric measurement and the antimony trichloride (Carr-Price or trifluoracetic acid) techniques for vitamin A **[134]** Such processes are still helpful, for instance in developing nations without access to contemporary equipment, but they take a lot of time, are challenging to automate, and are susceptible to interferences (like those caused by carotenoids, for example). In addition, the Carr-Price and trifluoracetic acid-based techniques use extremely corrosive chemicals and are not very sensitive, necessitating a high sample volume. Techniques based on HPLC Before and during HPLC, it's critical to reduce oxidative degradation, especially for oxygen-sensitive carotenoids. This is often accomplished by incorporating an antioxidant in the sample and BHT in the eluting solvent, such as butylated hydroxytoluene (BHT) or ascorbic acid. In addition, a protein denaturant—often ethanol—is typically introduced along with an internal standard of known concentration that can be isolated from other peaks and is absent from normal blood. A practical internal standard for one or more artificial carotenoids, like echinenone, may be employed as an internal standard for the carotenoid peaks. The carotenoid chromatogram's complexity, however, favors a standard level of difficulty. The lipid fraction is separated by extraction into a hydrocarbon such as hexane or heptane after the internal standard has been added

to the ethanol solution. For the HPLC separation, the extract is typically re-dissolved in the mobile phase after being evaporated under nitrogen, and then it is injected. If possible, a wavelength-switching capability is used to monitor absorbance at two or three wavelengths in the ultraviolet (UV) and visible range for the best component detection. A quantitative response factor is produced for each drug of interest by recalibrating the system with pure or semi-pure standards of known concentration (often based on their extinction coefficients) and purity (based on their chromatographic absorbance pattern). Most of the circulating vitamin A in individuals who are not vitamin A-overloaded and who have not recently consumed a significant amount of vitamin A is in the form of non-esterified (free) retinol, which is bound to the circulating carrier protein retinol-binding protein and further complexed with transthyretin (thyroxine-binding pre-albumin). In a chromatogram of a lipid extract of serum, the highest vitamin A-containing peak is often free retinol. For several months, serum or plasma samples that are utilized for vitamin A analysis can be kept at - 20°C, or better still, at -80°e. Since many carotenoids cannot be haemolyzed, they must also be light-protected **[133-139].** The key advantages of this method are its speed, use of small amounts of samples in microlitres, and relative freedom from interfering contaminants **[132,140,141],** this is especially significant given the current global interest in the relationship between micronutrients, nutrition, and many diseases patterns **[142]**. Retinol (vitamin A) and its synthetic derivatives are significant from the perspective of their potential utility as immunomodulatory agents. The fact that serum has a higher content of vitamin A could indicate that the vitamins are transported from the circulation to the seminal plasma via transudates. Similar to this, there are significant implications for the greater serum and seminal levels of vitamin A in males with normal sperm parameters compared to those with sperm dysfunction. Such males may have mild oxidative stress or a normal

functioning antioxidant scavenging system. On the other hand, low levels of vitamin A in the serum or seminals of males with sperm failure may be a sign of reduced antioxidant activity as a result of increased oxidative stress from lipid peroxidation, which is typically present in these conditions or leukocytosis **[124,127].** Therefore, it is tempting to hypothesize that the ratio of a-tocopherol to retinol in semen may be a more significant measure or biomarker of sperm activity than the absolute vitamin A levels in the seminal fluid. Especially among males who consume a diet high in vitamin A, the involvement of humoral and cellular interaction in infertility has continued to draw a lot of attention **[144,145].** Reactive oxygen species are produced by faulty spermatozoa and seminal leucocytes due to lipoprotein peroxidation, which is reduced by antioxidants having scavenging activity **[127,145],** these vitamins might be able to maintain sperm quality. When humans get vitamin supplements containing the vitamin's precursor, b-carotene, it is known to improve both T- and B-cell mitogen responsiveness and to encourage the growth of the circulating T-cell (CD+ 3) and helper T-cell (CD+ 4) subsets **[143].** In conclusion, HPLC is a quick and reliable method for estimating retinol levels simultaneously in serum and seminal fluid. These vitamins are present in higher amounts in men with normal sperm parameters compared to those with defective sperm, and their levels are higher in serum than in semen.

11. Conclusion

Vitamin A is crucial for male and female reproduction, aiding the immune system and protecting reproductive organs from oxidative stress. It is primarily found in beta-carotene and retinol, with retinoic acid potentially contributing to male fertility. Deficiency in vitamin A can damage the seminiferous epithelium, preventing spermatogenesis. Retinoic acid induces the expression of genes involved in human spermatogenesis, such as STRA8 and REC8, which strengthen the blood-testis barrier. Sperm DNA damage and lipid peroxidation are caused by oxidative stress in the reproductive system, potentially causing male infertility. Carotenoids, including lycopene, protect against free radical damage and have strong double bonds. High tissue absorption increases the risk of chronic diseases, while lycopene and beta-carotene have inverse associations with cardiovascular disease and malignancies. For mitosis to proceed to the final stage of meiosis, spermatogenesis must be activated and modulated by RA. In the process of spermatogenesis, RA appears to have a crucial function at the molecular level. Serum carotenoid antioxidant levels (vitamin A and beta-carotene), can be used to determine how well sperm is protected from free radicals by their environment. Vitamin A can be administered to reduce oxidative stress and boost the likelihood of conception if idiopathic infertility is diagnosed and found to be caused by oxidative stress. Techniques for determining retinol levels include immunoassay technology, reverse phase open column chromatography, and high-performance liquid chromatography.

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