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Retinoic Acid Determines the Fate of Spermatogonia

Review Article

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ABSTRACT

In this review, we aim to examine the effect of extrinsic and intrinsic factors on spermatogenesis process taking into account a complex signaling pathway. Primarily, it is suggested that retinoic acid (RA) has a vital function in spermatogenesis process and it is considered to be essential for completion of spermatogonia into mature spermatozoa. In the present review, the key effects of retinoic acid (RA) on spermatogenesis process were evaluated in the perspective of studies done, particularly at molecular level. Thus, this review will provide re look the spermatogenesis process in an outlook of new studies.

KEY WORDS retinoic acid, spermatogenesis, spermatogonia, testis.

INTRODUCTION

The importance of vitamin A on reproduction was firstly investigated in female and male rats by Thompson *et al.* (1964). In that study, it has been proposed that retinol is essential to male or female reproductive functions. Nevertheless, retinoic acid (RA) has not been essential. At the same time, Van Beek and Meistrich (1992) reported that spermatogenesis can be re-initiated with retinol treatment rather than RA treatment in retinol deficient rats.

Deficiency of vitamin A causes the testicular dysfunctions and development abnormalities before and after birth in rats and mice (Morales and Griswold, 1987; Griswold *et al.* 1989; Lufkin *et al.* 1993; Ross, 1993; Kastner *et al.* 1996; Huang *et al.* 2001; Blomhoff and Blomhoff, 2006; Ohta *et al.* 2010; Li *et al.* 2011; Griswold *et al.* 2012).

In fact, spermatogenesis is a complicated process and it is influenced from many extrinsic and intrinsic factors, and more importantly these factors are interacted with each other in a complex signaling pathway of spermatogenesis. RA is involved in pre meiotic germ cell function in embryonic testes and ovaries.

However, little is known about the mechanism by which vitamin A affects germ cell development based on sex differences in fetal period. It has been proposed that the Stra8 gene stimulated by RA has been found to be required for the transition germ cells into meiosis in both female and male cells. Indeed, *in vivo* and *in vitro* experiments have clearly demonstrated that RA induces mouse fetal testis to enter meiotic prophase (Oulad Abdelghani *et al.* 1996; Bowles *et al.* 2006; Ghyselinck *et al.* 2006; Kubova *et al.* 2006; Vernet *et al.* 2006a; Bowles and Koopman, 2007; Mc Lean *et al.* 2007; Trautmann *et al.* 2008; Zhou *et al.* 2008; Wright, 2010; Snyder *et al.* 2011). These key effects of RA on spermatogenesis process were evaluated in this review.

Retinoic acid metabolism

RA, an active metabolite of vitamin A, is largely obtained from retinol, but it is also produced from β-carotinoids relatively with low activity of cycle (Gaemers *et al.* 1996; Napoli, 1996; Zhai *et al.* 2001). Most RA synthesis occurs in situ in the testis, and the little remaining RA is delivered by plasma (Kurlandsky *et al.* 1995; Pfahl and Chytil, 1996). RA metabolism is essentially carried out in the myoid cells containing

high amounts of cellular retinol binding proteins (CRBP). These proteins bind retinol with high affinity. Hence, myoid cells are a barrier in the regulation of retinol's metabolism and then retinol bound into other transport proteins, such as retinol binding protein (RBP) and transthyretin (TTR), and secreted it as a complex formed with a new RBP, in the direction of the sertoli cells (Davis and Ong, 1995; Maekawa *et al.* 1996; Livera *et al.* 2002; Vernet *et al.* 2006b). It is generally thought that synthesis of RA from circulating of retinol and the enzymes associated with its synthesis are controlled by sertoli cells. Sertoli cells are also the main site of retinol storage, indicating that it expresses the lecithin-retinol acyltransferase (LRAT), which allows the esterification of retinol (Blaner *et al.* 1987; Eskild *et al.* 1991; Deltour *et al.* 1997). However, germ cells can synthesize their own RA, expressing the LRAT, especially at the spermatid stage (Schmitt and Ong, 1993). RA has also the cellular RA binding proteins, CRABP I and CRABP II, localized in the cytoplasm (Porter *et al.* 1985; Blaner *et al.* 1987; Rajan *et al.* 1991; Zheng *et al.* 1996). These proteins transmit RA to the nucleus and are responsible to induce the RA receptors, except for peritubular cells (Ruberte *et al.* 1992; Delva *et al.* 1999). Nevertheless, Lampron *et al.* (1995) showed that CRABP I and CRABP II are not critically involved in the RA signaling pathway. They detected that CRABP $I^{-/-}$ and CRABP $II^{-/-}$ (knock out foot his proteins) double mutant mice appear to be essentially normal because they are in contrast to abnormalities associated with vitamin A deficiency. As for the degrading of RA, it is catalyzed by CYP26s enzymes, which they have a group of P450 enzymes that metabolize RA to inactive forms (Figure 1) (Deltour *et al.* 1997; Molotkov *et al.* 2002; Yashiro *et al.* 2004; Doyle *et al.* 2007; Mc Lean *et al.* 2007).

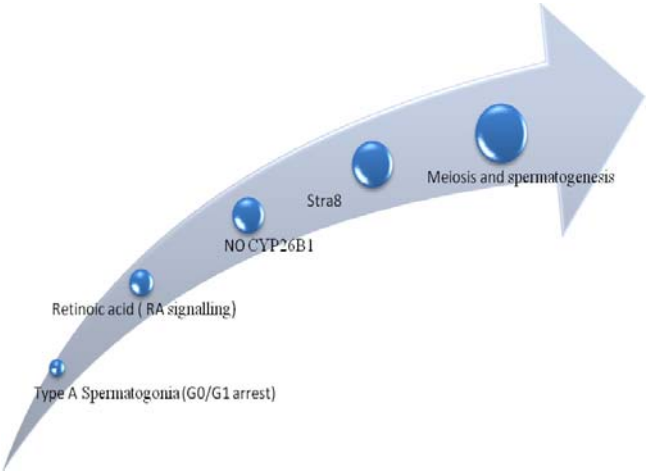

Figure 1 The effect of retinoic acid on spermatogenesis (Bowless and Koopman, 2007; Griswold *et al.* 2012)

Retinoic acid receptors

RA receptors include two main families. Each family comprises three classes of RA receptor (α, β and γ), each of them encoded by different genes.

These receptors are located in rat and mice testis, verifying types of cells and age of animals. Generally, these classes of receptors, including retinoid, express models alter according to the fetal or neonatal, and adult testis. Thus, it means that RA receptors have very complex mechanisms and their functions not only depend on cell type but also on the stage of testicular differentiation and the spermatogenic stage (Kim and Griswold, 1990; Davis and Lazar, 1993; Huang *et al.* 1994; Akmal *et al.* 1997; Akmal *et al.* 1998; Boulogne *et al.* 1999; Cupp *et al.* 1999; Dufour and Kim, 1999; Rouiller Fabre *et al.* 2003). In addition, it can imply that only some receptors, such as retinoic acid receptor α (RAR α) and retinoic × receptor β (RXR β), are important for testicular function or health of mice or rats (Lufkin *et al.* 1993; Kastner *et al.* 1996).

RAR α and RXR β ablation causes postnatal lethality similar to that saw in mice maintained on a Vitamin A deficient (VAD) diet as well as failure of spermatid release in germinal epithelium and the maturation of spermatozoa in epididymis (Lufkin *et al.* 1993; Kastner *et al.* 1996).

Russel *et al.* (1990) reported that the accumulation of lipid in sertoli cells could result from widespread phagocytesis of abnormal spermatids. However, in RXR β mutant mice it was observed lipid accumulation in sertoli cells of 29-day-old (Kastner *et al.* 1996). Conversely, lipid droplets in sertoli cells are not observed in any stages in the testis of RAR α mutant mice and in VAD rats or mice, even though there are similar anomalies as spermated release (Huang and Marshall, 1983; Lufkin *et al.* 1993).

Vernet *et al.* (2006b) further detected that spermation requires RXR β and RAR α heterodimers, but spermatogonia proliferation involves, independently of RXR, two distinct RAR mediated signaling pathways in both sertoli cells and spermatogonia.

RAR and RXR receptors can be expressed by RA ligand administration or injection. For example, all-trans-retinoic acid (ATRA) injection increases the expression of RAR α, RAR β, and RAR γ in mice testis (Gaemers *et al.* 1997). Similarly, administration of 9-cis-RA, natural for the RXRs, stimulated transiently mRNA expression of the nuclear RA receptor RAR β, suggesting a role for this receptor in the effects of retinoids on the differentiation and proliferation of type a spermatogonia (Gaemers *et al.* 1998a).

Treatment with a RA agonist and antagonist in rat testes influence the development in culture of leydig, sertoli and germ cells. Both RAR α and RAR β agonists mimic most of the effect of RA on the cultured neonatal rat testis. These effects can be reversed into antagonist behaviors of both RAR receptors (Livera *et al.* 2001). Besides, the effect of retinoids is not cumulative.

Therefore, it can imply differences between *in vivo* and *in vitro* experiments or the apparent cell-specific differences *in vitro* experiments.

In addition, the absence of the RXR γ occasionally does not seem to change the functions of the RXR α and RXR β. It is thought that RXR α is sufficient to perform most of the func-

tion of the RXRs in embryonic cells (Kastner *et al.*, 1996; Krezel *et al.* 1996).

Retinoic acid and testicular development

RA causes disorganization of the seminiferous cords in the foetus rat and also has multiple effects on development in the fetal and neonatal rat testis. Many studies demonstrate that RAR-selective agonists and all-trans RA completely inhibits seminiferous cord formation in 13-dpc (day point coitum) cultured rat testis.

At the same time, the effects of RA generally change with the developmental stage of the testis. For example, RA causes no disorganization of the semniferous tubules in 3-dp rat testis, but it causes the other disruptions, such as hyperplasia in its sertoli cells (Cupp *et al.* 1999; Livera *et al.* 2000; Livera *et al.* 2001; Sato *et al.* 2005). Livera *et al.* (2001) discovered that culture with RA for 3 days causes the disorganization of seminiferous tubules of the 14.5 dpc rat testis.

It was also reported that treatment with RA for 3 hours at the beginning of the culture is sufficient to prevent morphogenesis of the seminiferous cords in the developing rat testis (Marinos *et al.* 1994). In the same way, Huang *et al.* (2001) showed that retinoid treatment in mice has the adverse effect on morphogenesis at the early post-implantation stages of their gestation. In rat, however, CRABP and CRABP-II have positive effects on germ cells and embryonic cells, protecting from the effects of RA, interestingly (Zheng *et al.* 1996).

The effects of RA on gonocytes take place in a time-dependent manner (Livera *et al.* 2000; Livera *et al.* 2001; Lambrot *et al.* 2006). Livera *et al.* (2000) observed that in testes explanted on 18.5 dpc RA does not change the number of gonocytes, whereas it increases this number in rat testes explanted on 3 dpp (daily post partum). However, Boulogne *et al.* (1999) detected that in cultures of dispersed testicular cells from 3 dpp pups, RA has deleterious effect on the survival of the gonocytes and to a lesser extent on the somatic cells. Generally, it is thought that during the first fetal period RA has a negative effect on the testis, but in neonatal periods it supports the testicular development. On the other hand, the effect of RA is greatly influenced by culture conditions. For that reason, the different results can be greatly explained by different culture conditions for RA effects (Livera *et al.* 2000; Livera *et al.* 2001; Creemers *et al.* 2002). Moreover, origin of gonocytes is important to the culture outcome. Creemers *et al.* (2002) reported that the origin of isolated germ cells in germ cell cultures has strong impact on the culture outcome, showing that pre pubertal germ cells are twice as viable. They additionally showed that the addition of serum may improve the proliferation of the spermatogonial cells, but it could also be disadvantageous in the long-term.

Treated with RA, embryoid cells are affected considerably regarding the expression of genes associated with male germ cell lineage. Silva *et al.* (2008) demonstrated that optimal changes in gene expression were induced when cells were treated with RA, but the effects of RA occurs in a time- and concentration-dependent manner. Treating with both RA and testosterone, Silva *et al.* (2008) demonstrated that this combination in embryoid cells significantly increased Stra8 mRNA levels at 14 days. A similar result was demonstrated by Vernet *et al.* (2006a) in the mouse testis. In neonatal mice cultures without feeder cells, RA stimulates gonocyte DNA replication and differentiation in the cultured neonatal testes (Zhou *et al.* 2008). The RA metabolizing enzymes, CYB26A1 and CYB26B1, are believed to play important roles in protecting certain embryonic tissues from inappropriate RA signaling (Mc Lean *et al.* 2001). Nevertheless, little is known how RA inhibits germ cells from entering meiosis in the male fetuses. It was proposed that embryonic testes RA is most likely degraded by CYP26B1 enzyme, which belongs to CYP26 family. Hence, the Stra8 expression is inhibited and timing initiation of meiosis is postponed until after birth. These events regarding both production and degradation of RA occur outside germ cells, in somatic cells and tissues of embryo. In the RA environment, which is determined by ovaries and testes germ cells, it behaves according to sex differences. CYP26B1 is responsible for establishing a barrier to protect embryonic testes from inappropriate exposure to RA, thus maintaining germ cells in mitotic quiescence until meiosis, and it is required for spermatogenesis by maintaining the viability of germ cells during embryonic development. In the absence of CYP26B1, germ cell loss is also observed due to apoptosis. Consequently, this shows that Cyp26b1 (Figure 2) is significant for survival of germ cells (Abu Abed *et al.* 1998; Yamamoto *et al.* 2000; Ghyselinck *et al.* 2006; Kubova *et al.* 2006; Vernet *et al.* 2006a; Doyle *et al.* 2007; Mc Lean *et al.* 2007; Zhou *et al.* 2008).

Retinoic acid and spermatogenesis

Spermatogenesis can be reinitiated after vitamin A replacement in the VAD animals. It has been generally demonstrated that in vitamin A deficient rats and mice, almost all spermatogonia arrest in the G_1 phase of the cell cycle before the S phase of A_1 spermatogonia and the remaining spermatogonia are a spermatogonia and preleptotene spermatocytes. At days 7 and 21 after vitamin A replacement, spermatogenesis reinitiates and it is similar to that in normal testis. Interestingly, however, the degeneration occurs in the remaining spermatocyetes and most of them do not develop to spermatids (Griswold *et al.* 1989; Ismail *et al.* 1990; van Pelt and de Rooij, 1990; Van Pelt and de Rooij, 1991; Van Pelt *et al.* 1992; van Pelt *et al.* 1995; van Pelt *et al.* 1996; Gaemers *et al.* 1998a; Gaemers *et al.* 1998b; Ghyselinck *et al.* 2006). It is also proven that the deficiency of RA causes the dysfunction of somatic cells, especially in sertoli cells (Lufkin *et al.* 1993; Kastner *et al.* 1996; Akmal *et al.* 1998). By contrast, Mc Lean *et al.* (2007) detected that in CYP26B1 mutant mice, somatic cells are normal in the testis (Figure 2).

Doyle *et al.* (2007) showed that in tubules of RAR ∞ knockout mutant mice, germ cell degeneration took place in early meiotic prophase spermatocytes compared to the wild-

type tubules. This effect of RAR ∞ gene can be evidenced by transplanting the germ cells of RAR ∞ knockout mutant mice into wild type testis or vice versa.

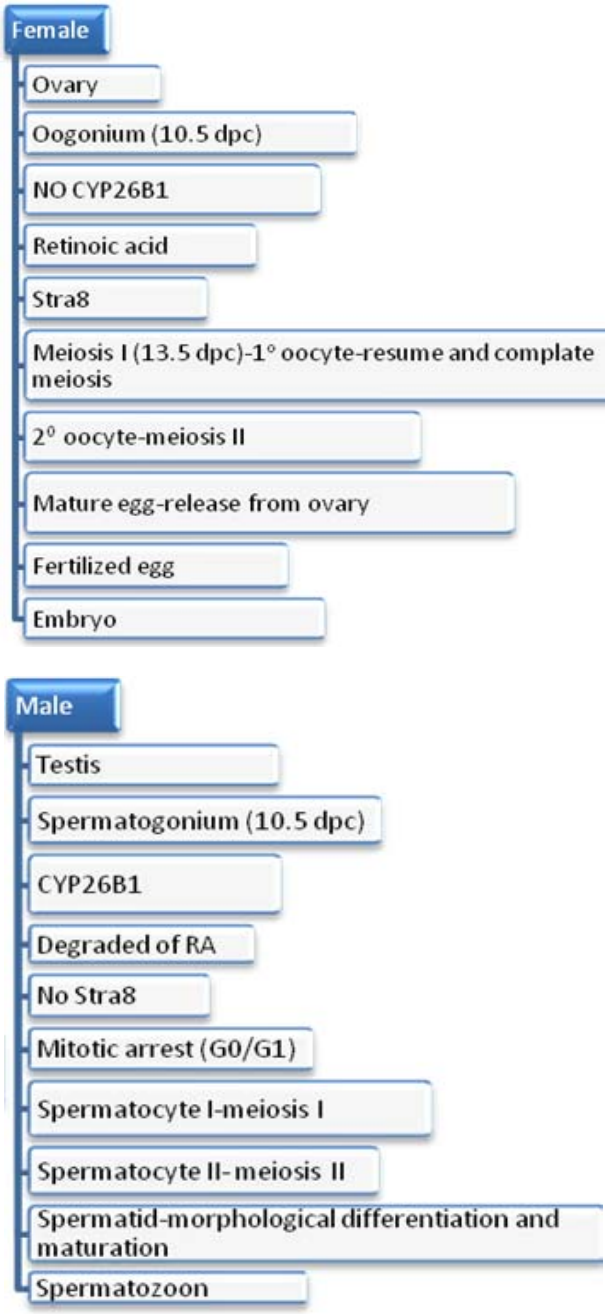

Figure 2 The effect of retinoic acid based on sex differences (Bowless and Koopman, 2007; Griswold *et al.* 2012)

But, germ cell loss is dependent on the stages of the spematogenic cycle. It was especially observed that germ cell loss is negligible in stages I-V, but severe after stages VIII and IX of the spermatogenic cycle. These findings show that RAR ∞ has a critical importance to promote the survival and development of early meiotic prophase spermatocytes.

Furthermore, RA is significant for spermatozoa maturation. Akmal *et al.* (1996) established that RAR α is expressed in epididymis. It is the highest in the proximal caput, low in the corpus and high again in the distal cauda, indicating the regional specificity.

In another study (Akmal *et al.* 1998), they detected that actions of RA occur in the sub-cellular levels instead of cellular. In fact, in VAD rats, cytoplasmic RAR α protein were expressed in the advanced germ cells despite the testicular degeneration. This finding also shows that the effect of RA takes places at nuclear levels (Ross, 1993; Akmal *et al.* 1998; Doyle *et al.* 2007).

In the absence of RA, testosterone secretion significantly declines in the fetal, developing and adult rat testes (Appling and Chytil, 1981; Livera *et al.* 2002). In a study (Livera *et al.* 2000) it was also confirmed that RA has a negative impact on testosterone secretion in the fetal and postnatal rat testis in culture. This effect of RA takes place in a dose-dependent manner and it results from a decrease on P450c17 mRNA levels and in LH-stimulated cAMP production rather than in a diminution on leydig cells. Fetal leydig cells may be specific cells and different than neonatal leydig cells. More interestingly, several authors (Rouiller Fabre *et al.* 2003; Zhang *et al.* 2004) reported that fetal testis has a specific physiological effect which largely differs from that of the immature or adult testis.

Summarily, RA reduces the expression of LH receptors and thus LH-stimulated testosterone decreases rather than basal testosterone (Chaudhary *et al.* 1989; Boulogne *et al.* 1999; Cupp *et al.* 1999; Livera *et al.* 2004).

One possible explanation is that LH signaling is not involved in the fetal testis. Zhang *et al.* (2004) showed that LH signaling is essential for the expression of genes with endocrine function in adult leydig cells rather than fetal leydig cells in LH receptor knockout mice.

Generally, the effect of RA on FSH takes place via sertoli cells. Galdieri and Nistico (1994) detected that in cultured rat sertoli cells FSH induced cAMP production of sertoli cells which it is reduced treating by retinol or RA. For this reason, it can be stated that retinoids modulate FSH action on cultured rat sertoli cells and decrease cAMP production. Similarly, Braun *et al.* (2000) also observed that ATRA induced the nuclear localization of RAR protein expression whereas FSH conversely suppressed the ATRA induced nuclear localization. In E13 rat testis organ cultures, RA inhibits FSH and epithelial growth factors (EGF) effects. This effect of RA occurs in dose-dependent manner. It was thought that the inhibitory actions of retinoids may in part be mediated through increased expression of T (Transforming)-GFβ (growth factor-beta) isoforms (Cupp *et al.* 1999). Matsuda *et al.* (2005) also showed that depletion of retinoids from the diet leads to an up-regulation of CREM (cAMP-responsive element modulator) expression in adult testes. Moreover, they stated that ATRA specifically represses the expression of the activator spliced variant of CREM. Conversely, Guo *et al.* (2001) reported that FSH induces the synthesis of retinol esters. This demonstrates that both FSH and RA are important regulator factors of testis

physiology. Therefore, the relationship between FSH and RA has a significant implication in understanding the mechanism of spermatogenesis.

CONCLUSION

As a result, it has been established that RA, the metabolite of vitamin A, has a significant impact for many functions of body. One of the most important roles of RA is on spermatogenesis process, which is considered indispensable in order to complete spermatogonia into mature spermatozoa for the developing of adult testis. In the embryonic period, RA takes other important role by inhibiting entering meiosis of male germ cells until after birth and protecting germ cells. In contrast, deficiency of RA causes the degeneration of germinal epithelium by arresting spermatogenesis and abnormalities of spermatozoa. In general, signaling pathway of RA is very complex and it has many aspects that are poorly understood regarding the metabolism. Furthermore, the mechanisms of retinoids' action as well as their role in physiology and pathology are other issues to be considered in future studies. Finally, it can be summarized that effect of RA in reproduction occurs in an organ-tissues-cell-stage manner, and this can be evidenced with the RA administration.

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