

## The role of retinoic acid receptors and their cognate ligands in reproduction in a context of triorganotin based endocrine disrupting chemicals

MACEJOVA D, TOPOROVA L, BRTKO J

*Institute of Experimental Endocrinology BMC, SAS, Bratislava, Slovak Republic*

*E-mail: ueenmace@savba.sk*

Retinoic acid (RA), an active form of vitamin A, regulates the embryonic development, male and female reproduction and induces important effects on the cell development, proliferation, and differentiation. These effects are mediated by the retinoid (RAR) and rexinoid nuclear receptors (RXR), which are considered to be a ligand-activated, DNA-binding, trans-acting, and transcription-modulating proteins, involved in a general molecular mechanism responsible for the transcriptional responses in target genes. Organotin compounds are typical environmental contaminants and suspected endocrine disrupting substances. They may affect processes of reproductive system in mammals, predominantly via nuclear receptor signaling pathways. Triorganotins, such as tributyltin chloride (TBTCl) and triphenyltin chloride (TPTCl), are capable to bind to RXR molecules, and thus represent potent agonists of RXR subtypes of nuclear receptors not sharing any structural characteristics with endogenous ligands of nuclear receptors. This article summarizes selected effects of biologically active retinoids and rexinoids on both male and female reproduction and also deals with the effects of organotin compounds evoking endocrine disrupting actions in reproduction.

**Key words:** retinoic acid, retinoic acid receptors, retinoid X receptors, reproduction, organotin compounds, endocrine disruptor

Retinoic acids are vitamin A (retinol)-derived, nonpeptidic, small lipophilic molecules that serve as ligands for two families of nuclear receptors, the retinoic acid (RA) receptors (RARs) and the retinoid X receptors (RXRs). Retinoids are involved in the complex arrangements of physiological and developmental responses in many tissues of higher vertebrates that include embryonic development, vision, reproduction, bone formation, haematopoiesis, metabolism, growth and differentiation of a variety of cell types, apoptosis, and processes of carcinogenesis. It is well known that retinoids are also teratogens and their therapeutic doses are contraindicated during pregnancy (Brtko 2007). In mammals, excess of vitamin A leads to a loss of germ cells (Lamano

Carvalho et al. 1978), and vitamin A deficiency leads to an arrest in spermatogenesis at early meiosis (Akmal et al. 1998). Experimental studies have suggested that RA is critical for the initiation of meiosis (Bowles et al. 2006; Koubova et al. 2006; Doyle et al. 2007) and spermiogenesis (Chung et al. 2005; Doyle et al. 2007). Some tissues contain small cytosolic proteins that specifically bind RA (rat brain, skin, testis, adrenals) (Bailey and Siu 1990). These are cellular retinoic acid-binding protein (CRABP) and cellular retinoic acid-binding protein II [CRABP(II)], well described members of a large family that includes a number of fatty acid-binding proteins (Bass 1993). The roles for this family of proteins are thought to help in the solubilization of their hydrophobic ligands and in some

cases, particularly for the retinoid-binding proteins, to direct metabolism of the ligand. Zheng *et al.* (1999) have shown that the pattern of CRABP(II) messenger RNA and protein expression correlated with the appearance of corpora lutea and the rise in progesterone production in rat as the corpora lutea get developed (Zheng *et al.* 1999). The level of RA present in a given tissue is finely tuned by the balance between its synthesis by RALDHs and its oxidative degradation by the following cytochrome P450 enzymes: cytochrome P450, family 26, subfamily a, polypeptide 1 (CYP26A1); cytochrome P450, family 26, subfamily b, polypeptide 1 (CYP26B1) – an enzyme that degrades the potent morphogen RA); and cytochrome P450, family 26, subfamily c, polypeptide 1 (CYP26C1) (Duester 2008; Griswold *et al.* 2012).

The origin of RA in the urogenital system differs in various species. In humans, RA is produced directly in fetal gonads (Childs *et al.* 2011). In mouse, RA is produced in mesonephroi and diffuses to the gonads. However, the meiotic entry is triggered exclusively in ovaries. In testes, RA is degraded, most probably in Sertoli and Leydig cells, and this action prevents meiotic entry (Bowles *et al.* 2006; Koubova *et al.* 2006; Kashimada *et al.* 2011). Also in birds, RA is synthesized in the gonads (in the ovarian cortex as well as the testis cords) and it is degraded by Sertoli cells (Smith *et al.* 2008; Piprek *et al.* 2013).

The RA:RAR/RXR complex binds to RA response elements (RAREs) in target genes, recruiting corepressors or coactivators and thereby inducing or repressing the transcriptional activity. Huang *et al.* (2015) have identified the genes involved in the regulation of RAR signaling pathway (ESR1, CYP26A1, TRIM16) and retinol metabolism-related enzyme genes (DHRS3, CYP2C9, CYP26A1) that were highly expressed in porcine endometrium during pregnancy. It has been found that CYP26A1 might block the adverse effect of the RA in order to promote the successful mouse embryo implantation (Ma *et al.* 2012). These results have shown that, except the progesterone and estrogen, the interaction between the RA and estrogen signaling may be also important for the embryo-maternal communications and endometrium remodeling during the early pregnancy.

### **Sertoli cells: the role of retinoic acid and retinoic acid receptors**

Testicular function is influenced by both the endocrine (extra-testicular) and paracrine (intratesticular) factors. Maintenance of normal spermatogenesis is dependent on the anterior pituitary hormones,

luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which are synthesized and secreted under control of the hypothalamic gonadotropin-releasing hormone (GnRH). Signals from the gonadotropins exert directly on the Sertoli cells, which regulate spermatogenesis. LH and FSH signal through the luteinizing hormone receptor (LHR) and follicle-stimulating hormone receptor (FSHR), which are expressed by the Leydig and Sertoli cells, respectively. The paracrine regulation of spermatogenesis is provided by steroids, such as testosterone and estradiol, secreted by Leydig cells and by proteins such as inhibin and activin, a member of the TGF $\beta$  superfamily, synthesized by Sertoli cells (Cooke and Saunders 2002). Interactions between Sertoli cells and germ cells through physical interaction and the secretion of signaling molecules are essential for the healthy progression of spermatogenesis (Sylvester and Griswold 1994). Inhibin primary negatively regulates the FSH secretion from the pituitary gland, whereas the activin has been proposed to affect germ cell maturation at the step when gonocytes differentiate into the spermatogonia (de Kretser *et al.* 2001).

It has been shown that vitamin A deficient (VAD) animals are infertile (McCarthy and Cerecedo 1952) and this condition can be reversed by retinol (Griswold *et al.* 1989). Basal serum FSH and LH levels in VAD rats were higher than those of controls (Huang *et al.* 1985). Moreover, a synergistic effect of vitamin A and FSH on differentiation of the testicular germ cell has been observed in the adult cryptorchid testis, which only consists of an undifferentiated spermatogonia and Sertoli cells (Haneji *et al.* 1984). RA, biologically active form of vitamin A, is primarily synthesized by the Sertoli cells in the testis (Deltour *et al.* 1997). RA is required for the differentiation of Sertoli cells, proliferation of spermatogonia, the initiation of meiosis, and maturation of spermatids (reviewed in Santos and Kim 2010). Nourashrafeddin (2015) has suggested that gonadotropins may trigger the differentiation of spermatogonia and their meiotic entry through regulation of RA signaling in the seminiferous tubules within the testis and provide a novel working hypothesis on the mechanisms of gonadotropins to control spermatogenesis via RA, which is considered to be responsible for the cyclic differentiation of germ cells in the adult testis and the continual production of sperm.

In the Sertoli cells, all retinoid receptors are expressed, whereas RAR $\gamma$  and RXR $\beta$  are not expressed in the germ cells (Dufour and Kim 1999). The biological effect of RA is mediated through RAR $\alpha$  partnering with RXR $\gamma$  in germ cells and RXR $\alpha$  (Dufour and

Kim 1999). In adult mouse testes, RAR $\alpha$  is localized in the nuclei of Sertoli cells, spermatogonia, preleptotene and pachytene spermatocytes, and round and elongating spermatids.

RAR $\alpha$  plays an essential role in the regulation of germ cell development during the spermatogenesis (Akmal et al. 1998; Law 2013). RAR $\alpha$ -null animals have high neonatal mortality and exhibit male infertility phenotype. The surviving males have depleted germ cells and vacuolization in the testis (Doyle et al. 2007), whereas RAR $\beta$  and RAR $\gamma$  gene KO mice did not show any testicular phenotypes (Luo et al. 1995). Transplantation studies have shown that RAR $\alpha$  in germ cells is responsible for the colonization and proliferation of germ cells in the basal area of the seminiferous tubules (Doyle et al. 2007). On the other hand, RAR $\alpha$  in Sertoli cells were needed for the progression of germ cells through meiosis (Doyle et al. 2007). RAR $\alpha$  has been shown to be important for Sertoli cell differentiation (Walker 2003) and in the synchronization of the stages of the spermatogenic cycle (Chung et al. 2005). RAR $\alpha$  was positively regulated by protein kinase C and MAPK (Braun et al. 2002). RA and FSH are important proliferation and differentiation factors for Sertoli cells. FSH inhibited the nuclear localization of RAR $\alpha$ , leading to down-regulation of RAR $\alpha$  transcriptional function via cAMP and protein kinase A (Santos and Kim 2010) as RAR $\alpha$  has two PKA consensus sites in the ligand-binding domain (Braun et al. 2000). Therefore, FSH can stimulate Sertoli cell mitosis before puberty via controlling RAR $\alpha$ . Thereafter, as FSH levels decrease around puberty (Eskola et al. 1993), RAR $\alpha$  is able to translocate to the nucleus (Dufour and Kim 1999) and may participate in the switch from Sertoli cell proliferation to differentiation (Santos and Kim 2010).

#### **Granulosa cells: the role of retinoic acid and retinoic acid receptors**

Early studies have shown the presence of vitamin A in the ovary and its fluctuation in serum during the menstrual cycle, indicating that vitamin A may play a role in the ovarian function (Laurence and Sobel 1953). In humans, the role of RA in meiosis has been demonstrated only in the ovary (Childs et al. 2011; Griswold et al. 2012). Moreover, recent studies have shown the presence of all RXR isoforms in the mammalian ovary (Tatone et al. 2016).

Human ovarian granulosa cells undergo a complex differentiation process during the growth and maturation of the ovarian follicles (Richards 1980).

This process depends on the sequential effects of the two principal gonadotropins, FSH and LH. FSH acts on early antral follicles to stimulate growth, steroidogenesis, and the expression of cell surface LH receptors, which mediate the granulosa cell ability to respond to circulating LH. Subsequently, LH, in synergy with FSH, acts on the FSH-stimulated follicles to maintain growth and estradiol production and leads to full development of the dominant follicle, the only follicle reaching the preovulatory stage. Finally, the LH triggers ovulation and conversion of the residual follicle into a corpus luteum that, in turn, produces progesterone preparing the endometrium for a possible implantation (Tatone et al. 2016). It has been shown that the ability of FSH to stimulate the induction of LHR in rat granulosa cells is mediated, at least in part, by cAMP, since exogenously added cAMP or other agents that increase intracellular levels of cAMP mimic the action of FSH (Minegishi et al. 2000). The presence of RARs in ovarian cells, including granulosa and some luteal cells, indicate that these cells would also be targets for RA (Zhuang et al. 1994). These data have suggested that RA may regulate the ovarian function by autocrine and/or paracrine action. In rat granulosa cells, RA (0.1 nM) and retinol (10 nM) each synergistically enhance the ability of FSH to induce LHR and stimulate the formation of cAMP and progesterone. At higher concentrations, both retinoids inhibited these effects of FSH in rat granulosa cells. Minegishi et al. (2000) have shown that the receptor depletion by RA is concentration-dependent and RA (1 nM) abolished the effect of FSH on LHR mRNA in rat granulosa cells. This study has provided evidence for a down-regulation of the LHR when RA is added to granulosa cells in the presence of FSH. The response of LH-R protein and LH-R mRNA to cAMP analogs was inhibited by RA in granulosa cells in this experiment, suggesting that RA diminished the action of FSH at sites distal to cAMP generation in the granulosa cells. The observed inhibition of mRNA levels of LHR by RA may be the result of decreased LHR gene transcription and/or mRNA stability.

In mice, RA and a CYP26 inhibitor stimulated granulosa cell proliferation in a dose-response manner. It has been shown that RAR-mediated signaling is involved in both RA- and activin-induced granulosa cell proliferation (Kipp et al. 2011). Kipp et al. (2011) have provided a new insight into the mechanisms of activin action in the ovary and have suggested CYP26B1 and RA to be novel candidates for regulating postnatal follicle formation and development. To examine signaling mechanisms involved in the stimu-

latory effect of RA, Kipp *et al.* (2011) have treated the mouse granulosa cells with RA in the presence of the pan-RAR inhibitor AGN193109 (Johnson *et al.* 1999). AGN193109 completely blocked the stimulatory effect of RA on granulosa cell proliferation, suggesting that the effect was mediated through RARs. Because activin stimulates mouse granulosa cell proliferation and also suppresses Cyp26B1 expression, some of the proliferative effects of the activin may be mediated by decreased Cyp26B1, leading to increased RA levels.

### Retinoic acid and initialization of meiosis

It has been shown that RA can stimulate the expression of the premeiotic marker gene *Stra8* (stimulated by RA gene 8) and meiosis in mice in both sexes (Griswold *et al.* 1989). *Stra8* was first described as one of the group of RA-responsive genes (Oulad-Abdelghani *et al.* 1996) and deletion of *Stra8* resulted in the prevention of the meiosis in the germ cells of both sexes (Baltus *et al.* 2006). RA appears to be present in both male and female embryonic urogenital ridges. In the mouse ovary, induction of *Stra8* in fetal germ cells expressing *Dazl*, an intrinsic factor, is required for meiotic DNA replication and the subsequent events of meiotic prophase (Baltus *et al.* 2006; Lin *et al.* 2008). In mouse testes, RA action and the subsequent entry of gonocytes into meiosis in the embryonic male is inhibited by the presence of the enzyme cytochrome P450, family 26, subfamily b, polypeptide 1 (CYP26B1) (Bowles *et al.* 2006; Koubova *et al.* 2006). CYP26B1 degrades RA into metabolites, some of which are inactive. If CYP26B1 is inhibited in mouse embryonic testes in culture or if the gene encoding CYP26B1 is ablated, *Stra8* mRNA is synthesized in male mouse germ cells and meiosis is initiated (Bowles *et al.* 2006; Koubova *et al.* 2006; MacLean *et al.* 2007). After birth, RA induces *Stra8* in testicular germ cells, leading to meiotic initiation (Koubova *et al.* 2006; Anderson *et al.* 2008). Induction of *Stra8* in the embryonic male germ cells is sufficient to induce the synthesis of downstream markers of meiosis, such as synaptonemal complex protein 3 (SCP3) and the meiosis-specific recombinase DMC1 (Bowles *et al.* 2006). Therefore, the expression of *Stra8* is necessary for germ cells to enter into meiosis and is an excellent marker for the action of RA.

However, Koubova *et al.* (2014) have discovered that RA activates meiosis in two independent ways, both requiring *Dazl* expression in the germ cells. It has been shown that germ cells in *Stra8*-deficient murine fetal ovaries express *Rec8* (Baltus *et al.* 2006), encoding a meiosis-specific component of the cohe-

sin complex. *Rec8* is required for completion of sister chromatid cohesion, proper synapsis, and chiasmata formation (Bannister *et al.* 2004). A chromatin immunoprecipitation-sequencing (ChIP-Seq) study in embryonic stem cells identified RAR binding sites in both *Stra8* and *Rec8* promoter regions, suggesting that *Stra8* and *Rec8* may be regulated by RA directly (Oulad-Abdelghani *et al.* 1996; Mahony *et al.* 2011). However, in the same study, *Dmc1*, which is dependent on *Stra8*, does not show RAR binding sites, consistent with *Stra8* and *Rec8* being regulated directly, unlike *Stra8*'s downstream targets (Koubova *et al.* 2014).

It has also been suggested that the actual physiological role of RAR antagonists may be different from their reported functions because these compounds may exert nonspecific effects on other receptors (Kumar *et al.* 2011), based on a known case involving the RAR antagonist Ro 41-5253 (Schupp *et al.* 2007). This is a fair point, although the two pan-RAR antagonists that have been shown to inhibit meiosis are BMS-204493 and AGN193109, not Ro 41-5253 (Bowles *et al.* 2006; Koubova *et al.* 2006).

### Retinoic acid and primordial germ cells

In mouse, germ cell formation begins *in utero* with primordial germ cells (PGCs), precursor cells developing into ova and spermatozoa that form around embryonic day 6.25 (E6.25) and migrate from the proximal epiblast through the hindgut towards the genital ridge around E10.5 (Saitou and Yamaji 2012). From here, female (XX) and male (XY) PGCs enter two distinct pathways. While XX PGCs continue to proliferate until E13.5 and subsequently enter meiosis, XY PGCs are enclosed by testicular cords, become prospermatogonia or gonocytes around E12.5 (McLaren 1984) and are then arrested at G0-like state around E13.5. Gonocytes remain quiescent around E16.5 until shortly after birth (Vergouwen *et al.* 1991).

Koshimizu *et al.* (1995) have found that RA is a potent growth activator of mouse PGCs *in vitro* and promotes the proliferation of PGCs and slows down the degeneration of colonizing PGCs in culture as a mitogen both *in vitro* and *in vivo* (Koshimizu *et al.* 1995). It has been also found that RA acts as both a mitogen and a survival factor for germ cells during fetal mouse oogenesis *in vitro* and *in vivo* (Morita and Tilly 1999).

Mouse embryonic stem cells can be induced into primordial germ cell-like cells (PGCLCs) by RA and promote the self-renewal of PGCs *in vitro* (Geijsen *et al.* 2004; Eguizabal *et al.* 2009). It has been found

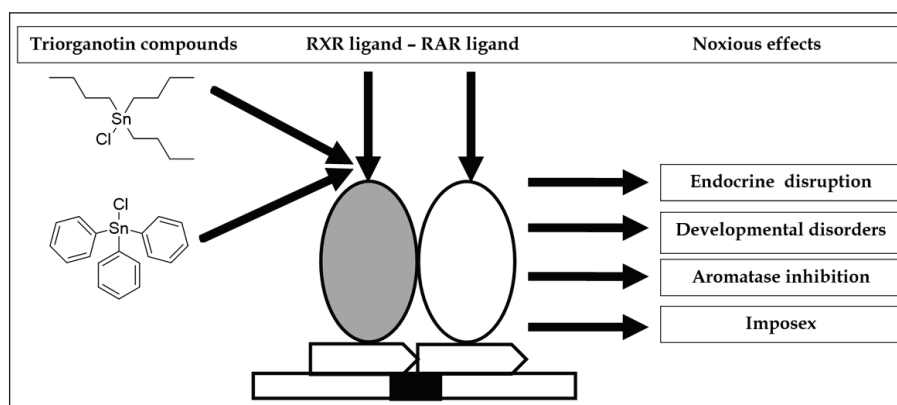
that cultures with the presence of RA attain PGC-like identity and continue to proliferate. Furthermore, the expression patterns of *Prmt5* and H3K27me3 in newly formed PGCs are similar to those of 11.5-dpc PGCs *in vivo* (Eguizabal et al. 2009). Tan et al. (2016) have found that RA induced the expressions of cell cycle-related genes. *CCND1* is an important regulator of G1-to-S phase progression (Dalton 1999), and *CDK2* can interact with cyclin E to drive cells through the G1-to-S transition and combine with cyclin A through the S-phase (Jirawatnotai et al. 2012). The increasing expressions of cell cycle-related genes suggested that cell cycle was affected after RA exposure. Meanwhile, the increase in the percentage of SSEA-1-positive PGCs suggested that RA could promote the proliferation of PGCs derived from mouse embryos *in vitro*, and *CCND1* and *CDK2* were also up-regulated after RA treatment, which was similar to PGCLCs cultured *in vitro* (Tan et al. 2016).

In chickens, Yu et al. (2011) have further verified that RA promotes the proliferation of chicken PGCs via the protein kinase C and PI3K/Akt signaling pathways. RA treatment increased the expressions of *CCND1*, *CCNE1*, *CDK6*, and *CDK2*, genes critical for G1-to-S phase progression in the cell cycle (Yu et al. 2011). Moreover, it has been confirmed that RA-treated chicken PGC populations have significantly increased proportion of S-phase cells (Yu et al. 2012; Tan et al. 2016).

### Organotin derivatives – RXR ligands – and reproduction

Organotin compounds are typical environmental contaminants and suspected endocrine disrupting substances (Brtko and Dvorak 2015). Humans are exposed to tributyltin (TBT), previously used as an antifouling paint in ships, mainly through fish con-

sumption. A remarkable breakthrough in the field came out with the recent findings that triorganotin compounds are agonists of RXR subtypes of NRs (Figure 1), not sharing any structural characteristics with any endogenous ligands of NRs. It has been shown that RXRa with its NR cysteine residue (C432) of helix H11 at the entrance to the ligand binding pocket was found to covalently interact with trialkyltin or triaryl tin (Grun 2014; Brtko and Dvorak 2015). After crossing the cell membrane, triorganotins could bind to NRs, which reside in the cytoplasm (e.g. glucocorticoid receptors) or in the nucleus (e.g. RXRs, PPARs) (Toporova et al. 2016). Upon ligand binding, triorganotin-NRs translocate to the nucleus where they form a complex triorganotin-NRs and co-activators, which binds to the response elements on the DNA and induces transcription of target genes, changes in the expression of some proteins, as well as mitochondrial and cell dysfunctions. TBT has been largely released into water from special paintings. At very low concentrations (pM or nM), TBT induced an irreversible sexual abnormality “imposex” in marine gastropods (Nakanishi 2008). Triorganotins have been suggested to have teratogenic and pathologic effects of on endocrine and reproductive system of mammals in both genders (Delgado Filho et al. 2011), and endocrine disrupting effects, such as induction of progesterone biosynthesis, effects on aromatase activity, and capability to induce transcriptional activity of thyroid hormone receptor (Brtko a Dvorak 2015; Illes et al. 2015; Hiromori et al. 2016). There are many reports regarding the biological effects of organotin compounds, which vary in their toxic effects on eukaryotes (reviewed in Delgado Filho et al. 2011). *In vitro* exposure of the human choriocarcinoma cell lines to TBT or triphenyltin (TPT) (300 nM) markedly decreased DNA and protein synthesis (Nakanishi et al. 2002). In the same concentration ranges, TPT



**Figure 1.** The effect of triorganotin-based endocrine disrupting chemicals on nuclear retinoid X receptor (RXR) and retinoic acid receptor (RAR) pathways.

also inhibited the catalytic activity of human aromatase (Lo *et al.* 2003) and other steroidogenic enzymes, affecting sexual development in male and female rats (Delgado Filho *et al.* 2011).

Organotin compounds can cross the placental barrier and accumulate in large quantities in the placenta and fetal tissues inducing morphological changes (Cooke *et al.* 2008; Delgado Filho *et al.* 2011). Kishta *et al.* (2007) have reported reduced number of gonocytes, Sertoli and Leydig cells of fetal testis after *in utero* exposure to TBTCl in pregnant rats. Growth retardation, delayed ossification of the fetal skeleton and reduced body weight were also detected in male offspring. Additionally, a reduced testosterone concentration as well as a significant delay in the age at preputial separation (Grote *et al.* 2009) has been found after *in utero* exposure to TBT of rats, without affecting the male reproductive system (Adeeko *et al.* 2003).

The placenta plays a vital role in the maintaining pregnancy. The production of steroid hormones, such as progesterone and estrogens, is a crucial function of the primate placenta. In human placenta, steroid biosynthesis is regulated by various steroidogenic enzymes. The enzyme 3 $\beta$ -hydroxysteroid dehydrogenase/isomerase (3 $\beta$ -HSD) catalyzes the conversion of 3-hydroxy-5-ene-steroids (dehydroepiandrosterone and pregnenolone) to 3-oxo-4-ene-steroids (androstenedione and progesterone) (Simard *et al.* 2005). Placental production of progesterone is required to protect the conceptus during gestation (Malassine *et al.* 2003), the ingestion of progestins (*i.e.*, natural and synthetic progesterone and testosterone derivatives that produce biologic effects similar to those of progesterone) during pregnancy is associated with an increased risk of hypospadias (Carmichael *et al.* 2005). Therefore, the developmental and reproductive toxicities of environmental contaminants known to have endocrine disrupting effects plausibly might involve placental 3 $\beta$ -HSD I in humans. It has been shown that TBT inhibits the catalytic activity of human 5 $\alpha$ -reductase I and II, rat 3 $\beta$ -HSD, and porcine 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD). TPT has been found to inhibit the catalytic activity of human aromatase, human 5 $\alpha$ -reductase II, 17 $\beta$ -HSD I and III (Doering *et al.* 2002; McVey and Cooke 2003; Ohno *et al.* 2005).

It has been shown that exposure of human choriocarcinoma JAr cells to nontoxic concentrations of both TBT and TPT enhanced 17 $\beta$ -HSD I mRNA transcription and enzyme activity in a dose-dependent fashion and enhanced aromatase activity (Nakanishi *et al.* 2006). Moreover, TBT and TPT act as

nanomolar agonists for both the RXR and peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (Hiromori *et al.* 2009). PPAR $\gamma$  regulates the transcription of genes by heterodimerizing with RXR and by binding to the PPAR response elements in the target gene promoter (Kliewer *et al.* 1992). The promotion of estrogen biosynthesis by the organotin compounds involves the activation of RXR rather than PPAR $\gamma$  (Nakanishi *et al.* 2006).

Hiromori *et al.* (2009) have found that some organotin compounds, including TBT and TPT, promote human chorionic gonadotropin (hCG) production (Hiromori *et al.* 2009). hCG is a luteotropic factor and its stimulation by hCG governs not only progesterone production in the corpus luteum during the first trimester, but also the testosterone production within the fetal testes. hCG is a crucial target gene of PPAR $\gamma$  in human placenta and its production and mRNA transcription is ligand-dependently controlled by PPAR $\gamma$  (Tarrade *et al.* 2001). To investigate the effects of RXR and PPAR $\gamma$  agonists on progesterone production and 3 $\beta$ -HSD I mRNA transcription, Hiromori *et al.* (2016) have treated JAr cells with LG (RXR agonist) or Rosi (PPAR $\gamma$  agonist). Both LG and Rosi enhanced progesterone production and 3 $\beta$ -HSD I mRNA transcription. These data suggest that the RXR and PPAR $\gamma$  signaling pathways may be involved in organotin-induced progesterone production in human placental cells.

Moreover, Hiromori *et al.* (2016) have show that triorganotins, TPrTCl, TBTCl, TChTOH, and TPTCl, significantly enhanced the progesterone production in a concentration-dependent manner in human choriocarcinoma JAr cells. Among metabolites of both TBTCl and TPTCl, DBTCl<sub>2</sub>, MPTCl<sub>3</sub>, and DPTCl<sub>2</sub> altered progesterone production, and the level of stimulation increased proportionally with the alkylation or arylation of these organotin compounds (tri- > di- > mono-). However, the presence of a fourth alkyl group on the tin atom decreased the potency of the organotin compounds, inducing the progesterone production, because tertbutyltin (TeBT) failed to stimulate this placental function at doses <100 nM. The organotin compounds that enhanced progesterone production also significantly increased the mRNA transcription of 3 $\beta$ -HSD I. All active organotins increased the mRNA transcription of 3 $\beta$ -HSD I in a concentration-dependent manner.

Several studies addressing the effect of TBT on male reproductive functions have been reported (Omura *et al.* 2001; Grote *et al.* 2004; Delgado Filho *et al.* 2011). Omura *et al.* (2001) have shown that dietary treatment with TBTCl resulted in decreased testis,

epididymis, ventral prostate, and body weight during two generation study in rats. In other studies, a significant decrease in the weight of the seminal vesicle and the weights of all reproductive organs has been reported in rodents (Grote et al. 2004). Prostate atrophy, as a consequence of aromatase inhibition, is also well known. Moreover, TBT induces morphological-functional changes in the testes, including vacuolization of seminiferous epithelium, delayed spermiation, spermatid retention into the germinative epithelium, and germ cell degeneration near the basement membrane. The frequencies of these impairments in male sex organs were greater and considered to be abnormal in the TBT-treated F2 generation in rats, although there was a dose-dependent increase in the serum testosterone levels of the rats fed by TBTCl diets and a decrease in serum estrogen levels in the F1 generation (Omura et al. 2001). In rats, the count of caudal epididymal and testicular sperm (Yu et al. 2003) and of homogenization-resistant spermatids decreased (Omura et al. 2001), and the motility, mean angular displacement, lateral head displacement, and dance of sperm from the vas deferens, were also reduced (Yu et al. 2003). Decreased serum concentrations of thyroxine and triiodothyronine were observed in another study, in association with extensive damage to the thyroid gland, and low expression of thyroid hormone receptor alpha in marine fish testes (Adeeko et al. 2003; Zhang et al. 2009).

A large number of studies have shown that exposure to organotin compounds can cause reproductive disruption in the female reproductive system of mammals (reviewed in Delgado Filho et al. 2011). Treatment with organotin compounds in pseudopregnant rats resulted in a decrease in uterine weight and serum progesterone levels, but ovarian weight, number of corpora lutea and estrogen levels remained at average levels. *In utero* exposure to high doses of TBT led to a decrease in the maternal weight gain and fetal weight, induced pre- and post-implantation losses (Nakanishi et al. 2005), provoked fetal toxicity (Itami et al. 1990), altered the anogenital distance of female pups on postnatal day

1 (Ogata et al. 2001), caused precocious completion of vaginal opening in postnatal females (Grote et al. 2009), reduced by about 45% the number of germ cells, and induced morphological-functional changes in the ovaries of fetal female rats (a large number of germ cells with pyknotic nuclei) (Kishta et al. 2007). In human choriocarcinoma cell lines, TBT and TPT increased levels of hCG secretion and aromatase activity in a dose- and time-dependent fashion following exposure to nontoxic concentration ranges (Nakanishi et al. 2002). In human choriocarcinoma JAr cells, trialkyltin and TPT enhanced 17 $\beta$ -HSD I mRNA transcription and enzyme activity in a dose-dependent fashion at nontoxic concentrations. (Nakanishi et al. 2006). However, in human granulosa-like cell line, TBT and TPT suppressed both aromatase activity and gene expression (Saitoh et al. 2001). Based on these results, it has been suggested that organotin compounds function as a powerful agonist for nuclear receptors rather than an aromatase inhibitor (Nakanishi 2008).

In conclusion, the effects of triorganotin compounds have been associated with gender-specific morphological-functional changes in mammalian reproductive organs. Organotin compounds have been shown to act potentially as inhibitors of steroidogenic enzymes (Delgado Filho et al. 2011) and proteasome (Saitoh et al. 2001), or enhancers of histone acetyltransferase (Nakanishi et al. 2006). Moreover, effects of triorganotin compounds have been shown on epigenetic regulation of gene expression (Stel and Legler 2015). Since recent studies have also shown that triorganotin compounds can exhibit anti-tumor activity (Tabassum and Pettinary 2006; Hunakova et al. 2015; Macejova et al. 2015), the further studies on triorganotin compound characteristics and action are essential.

### Acknowledgments

This work was supported by APVV-15-0372, APVV-15-0296, APVV-0160-11, and VEGA 2/0171/14 grants.

### References

- Adeeko A, Li D, Forsyth DS, Casey V, Cooke GM, Barthelemy J, Cyr DG, Trasler JM, Robaire B, Hales BF. Effects of in utero tributyltin chloride exposure in the rat on pregnancy outcome. *Toxicol Sci* 74, 407–415, 2003.
- Akmal KM, Dufour JM, Vo M, Higginson S, Kim KH. Ligand-dependent regulation of retinoic acid receptor alpha in rat testis: in vivo response to depletion and repletion of vitamin A. *Endocrinology* 139, 1239–1248, 1998.
- Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AM, Page DC. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc Natl Acad Sci USA*. 105, 14976–14980, 2008.

- Bailey JS, Siu CH. Unique tissue distribution of two distinct cellular retinoic acid binding proteins in neonatal and adult rat. *Biochim Biophys Acta* 1033, 267–272, 1990.
- Baltus AE, Menke DB, Hu YC, Goodheart ML, Carpenter AE, de Rooij DG, Page DC. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic dna replication. *Nat Genet* 38, 1430–1434, 2006.
- Bannister LA, Reinholdt LG, Munroe RJ, Schimenti JC. Positional cloning and characterization of mouse *mei8*, a disrupted allele of the meiotic cohesin *rec8*. *Genesis* 40, 184–194, 2004.
- Bass NM. Cellular binding proteins for fatty acids and retinoids: similar or specialized functions? *Mol Cell Biochem* 123, 191–202, 1993.
- Bowles J, Knight D, Smith C, Wilhelm D, Richman J, Mamiya S, Yashiro K, Chawengsaksophak K, Wilson MJ, Rossant J, Hamada H, Koopman P. Retinoid signaling determines germ cell fate in mice. *Science* 312, 596–600, 2006.
- Braun KW, Tribble WA, Griswold MD, Kim KH. Folliclestimulating hormone inhibits all-*trans*-retinoic acid-induced retinoic acid receptor nuclear localization and transcriptional activation in mouse Sertoli cell lines. *J Biol Chem* 275, 4145–4151, 2000.
- Braun KW, Vo MN, Kim KH. Positive regulation of retinoic acid receptor  $\alpha$  by protein kinase C and mitogen-activated protein kinase in Sertoli cells. *Biol Reprod* 67, 29–37, 2002.
- Brtko J. Retinoids, rexinoids and their cognate nuclear receptors: character and their role in chemoprevention of selected malignant diseases. *Biomed Pap Med Fac Univ Palacky (Olomouc, Czech Repub)* 151, 187–194, 2007.
- Brtko J, Dvorak Z. Triorganotin compounds-ligands for “rexinoid” inducible transcription factors: biological effects. *Toxicol Lett* 234, 50–58, 2015.
- Carmichael SL, Shaw GM, Laurent C, Croughan MS, Olney RS, Lammer EJ. Maternal progestin intake and risk of hypospadias. *Arch Pediatr Adolesc Med* 159, 957–962, 2005.
- Childs AJ, Cowan G, Kinnell HL, Anderson RA, Saunders PTK. Retinoic acid signalling and the control of meiotic entry in the human fetal gonads. *PLoS One* 6, e20249, 2011.
- Chung SS, Wang X, Wolgemuth DJ. Male sterility in mice lacking retinoic acid receptor  $\alpha$  involves specific abnormalities in spermiogenesis. *Differentiation* 73, 188–198, 2005.
- Cooke HJ, Saunders PT. Mouse models of male infertility. *Nature reviews. Genetics* 3, 790–801, 2002.
- Cooke GM, Forsyth DS, Bondy GS, Tachon R, Tague B, Coady L. Organotin speciation and tissue distribution in rat dams, fetuses, and neonates following oral administration of tributyltin chloride. *J Toxicol Environ Health A* 71, 384–395, 2008.
- Dalton S. Cell cycle control of chromosomal DNA replication. *Immunol Cell Biol* 76, 467–472, 1998.
- Delgado Filho VS, Lopes PF, Podratz PL, Graceli JB. Triorganotin as a compound with potential reproductive toxicity in mammals. *Braz J Med Biol Res* 44, 958–965, 2011.
- Deltour L, Haselbeck RJ, Ang HL, Duester G. Localization of class I and class IV alcohol dehydrogenases in mouse testis and epididymis: potential retinol dehydrogenases for endogenous retinoic acid synthesis. *Biol Reprod* 56, 102–109, 1997.
- de Kretser DM, Loveland KL, Meehan T, O’Bryan MK, Phillips DJ, Wreford NG. Inhibins, activins and follistatin: actions on the testis. *Mol Cell Endocrinol* 180, 87–92, 2001.
- Doering DD, Steckelbroeck S, Doering T, Klingmuller D. Effects of butyltins on human 5 $\alpha$ -reductase type 1 and type 2 activity. *Steroids* 67, 859–867, 2002.
- Doyle TJ, Braun KW, McLean DJ, Wright RW, Griswold MD, Kim KH. Potential Functions of Retinoic Acid Receptor A in Sertoli Cells and Germ Cells During Spermatogenesis. *Ann N Y Acad Sci* 1120, 114–130, 2007.
- Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell* 134, 921–931, 2008.
- Dufour JM, Kim KH. Cellular and subcellular localization of six retinoid receptors in rat testis during postnatal development: identification of potential heterodimeric receptors. *Biol Reprod* 61, 1300–1308, 1999.
- Eguizabal C, Shovlin TC, Durcova-Hills G, Surani A, McLaren A. Generation of primordial germ cells from pluripotent stem cells. *Differentiation* 78, 116–123, 2009.
- Eskola V, Nikula H, Huhtaniemi I. Age-related variation of follicle-stimulating hormone-stimulated cAMP production, protein kinase C activity and their interactions in the rat testis. *Mol Cell Endocrinol* 93, 143–148, 1993.
- Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, Daley GQ. Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature* 427, 148–154, 2004.
- Griswold MD, Bishop PD, Kim KH, Ping R, Siiteri JE, Morales C. Function of vitamin A in normal and synchronized seminiferous tubules. *Ann N Y Acad Sci* 564, 154–172, 1989.
- Griswold MD, Cathryn A, Hogarth CA, Bowles J, Koopman P. Initiating Meiosis: The Case for Retinoic Acid. *Biol Reprod* 86, 35, 1–7, 2012.



- Grote K, Stahlschmidt B, Talsness CE, Gericke C, Appel KE, Chahoud I. Effects of organotin compounds on pubertal male rats. *Toxicology* 202, 145–158, 2004.
- Grote K, Hobler C, Andrade AJ, Grande SW, Gericke C, Talsness CE, Appel KE, Chahoud I. Sex differences in effects on sexual development in rat offspring after pre- and postnatal exposure to triphenyltin chloride. *Toxicology* 260, 53–59, 2009.
- Grun F. The obesogen tributyltin. *Vitam Horm* 94, 277–325, 2014.
- Haneji T, Maekawa M, Nishimune Y. Vitamin A and follicle-stimulating hormone synergistically induce differentiation of type A spermatogonia in adult mouse cryptorchid testes *in vitro*. *Endocrinology* 114, 801–805, 1984.
- Hirumori Y, Nishikawa J, Yoshida I, Nagase H, Nakanishi T. Structure-dependent activation of peroxisome proliferator-activated receptor (PPAR) $\gamma$  by organotin compounds. *Chem Biol Interact* 180, 238–244, 2009.
- Hirumori Y, Yui H, Nishikawa J, Nagase H, Nakanishi T. Organotin compounds cause structure-dependent induction of progesterone in human choriocarcinoma Jar cells. *J Steroid Biochem Mol Biol* 155, 190–198, 2016.
- Huang HS, Dyrenfurth I, Gunsalus GL, Hembree WC. Effect of vitamin A deficiency upon gonadotropin response to gonadotropin-releasing hormone. *Biol Reprod* 33, 1176–1187, 1985.
- Hunakova L, Macejova D, Toporova L, Brtko J. Anticancer effects of tributyltin chloride and triphenyltin chloride in human breast cancer cell lines MCF-7 and MDA-MB-231. *Tumor Biol*, 2015. [Epub ahead of print].
- Illes P, Brtko J, Dvorak Z. Development and Characterization of a Human Reporter Cell Line for the Assessment of Thyroid Receptor Transcriptional Activity: A Case of Organotin Endocrine Disruptors. *J Agric Food Chem* 63, 7074–7083, 2015.
- Itami T, Ema M, Amano H, Murai T, Kawasaki H. Teratogenic evaluation of tributyltin chloride in rats following oral exposure. *Drug Chem Toxicol* 13, 283–295, 1990.
- Jirawatnotai S, Hu Y, Livingston DM, Sicinski P. Proteomic identification of a direct role for cyclin d1 in DNA damage repair. *Cancer Res* 72, 4289–4293, 2012.
- Johnson AT, Wang L, Gillett SJ, Chandraratna RA. High affinity retinoic acid receptor antagonists: analogs of AGN 193109. *Bioorg Med Chem Lett* 9, 573–576, 1999.
- Kashimada K, Svingen T, Feng CW, Pelosi E, Bagheri-Fam S, Harley VR, Schlessinger D, Bowles J, Koopman P. Antagonistic regulation of Cyp26b1 by transcription factors SOX9/SF1 and FOXL2 during gonadal development in mice. *FASEB J* 25, 3561–3569, 2011.
- Kipp JL, Golebiowski A, Rodriguez G, Demczuk M, Kilen SM, Mayo KE. Gene expression profiling reveals Cyp26b1 to be an activin regulated gene involved in ovarian granulosa cell proliferation. *Endocrinology* 152, 303–312, 2011.
- Kishta O, Adeeko A, Li D, Luu T, Brawer JR, Morales C, et al. *In utero* exposure to tributyltin chloride differentially alters male and female fetal gonad morphology and gene expression profiles in the Sprague-Dawley rat. *Reprod Toxicol* 23, 1–11, 2007.
- Kliwer SA, Umeson K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors, *Nature* 358, 771–774, 1992.
- Koshimizu U, Watanabe M, Nakatsuji N. Retinoic acid is a potent growth activator of mouse primordial germ cells *in vitro*. *Dev Biol* 168, 683–685, 1995.
- Koubova J, Menke DB, Zhou Q, Capel B, Griswold MD, Page DC. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc Natl Acad Sci USA* 103, 2474–2479, 2006.
- Koubova J, Hu YC, Bhattacharyya T, Soh YQ, Gill ME, Goodheart ML, Hogarth CA, Griswold MD, Page DC. Retinoic acid activates two pathways required for meiosis in mice. *PLoS Genet* 10, 1–9, 2014.
- Kumar S, Chatzi C, Brade T, Cunningham TJ, Zhao X, Duyster G. Sex-specific timing of meiotic initiation is regulated by Cyp26b1 independent of retinoic acid signalling. *Nat Commun* 2, 151, 2011.
- Lamano Carvalho TL, Lopes RA, Azoubel R, Ferreira AL. Morphometric study of the reversibility of testicle alterations in rats submitted to hypervitaminosis A. *Int J Vitam Nutr Res* 48, 316–324, 1978.
- Laurence PA, Sobel AE. Changes in serum vitamin A level during the human menstrual cycle. *J Clin Endocrinol Metab* 13, 1192–1199, 1953.
- Law SM. Retinoic acid receptor alpha in germ cells is important for mitosis of spermatogonia, spermatogonial differentiation and meiosis. Dissertation. Washington State University. 2013.
- Lin Y, Gill ME, Koubova J, Page DC. Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. *Science* 322, 1685–1687, 2008.
- Lo S, Allera A, Albers P, Heimbrecht J, Jantzen E, Klingmuller D, Steckelbroeck S. Dithioerythritol (DTE) prevents inhibitory effects of triphenyltin (TPT) on the key enzymes of the human sex steroid hormone metabolism. *J Steroid Biochem Mol Biol* 84, 569–576, 2003.

- Luo J, Pasceri P, Conlon RA, Rossant J, Giguere V. Mice lacking all isoforms of retinoic acid receptor beta develop normally and are susceptible to the teratogenic effects of retinoic acid. *Mech Dev* 53, 61–71, 1995.
- Ma JJ, Han BC, Yang Y, Peng JP. Retinoic acid synthesis and metabolism are concurrent in the mouse uterus during peri-implantation. *Cell Tissue Res* 350, 525–537, 2012.
- Macejova D, Bialesova L, Toporova L, Brtko J. Biological effects of selected triorganotin compounds – retinoid X receptor ligands in estrogen receptor negative MDA-MB-231 human breast cancer cells. *Toxicol Lett* 238S, S374, 2015.
- MacLean G, Li H, Metzger D, Chambon P, Petkovich M. Apoptotic extinction of germ cells in testes of Cyp26b1 knockout mice. *Endocrinology* 148, 4560–4567, 2007.
- Malassine A, Frenzo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 9, 531–539, 2003.
- McVey MJ, Cooke GM. Inhibition of rat testis microsomal 3beta-hydroxysteroid dehydrogenase activity by tributyltin. *J Steroid Biochem Mol Biol* 86, 99–105, 2003.
- Mahony S, Mazzoni EO, Mccuine S, Young RA, Wichterle H, Gifford DK. Ligand-dependent dynamics of retinoic acid receptor binding during early neurogenesis. *Gen Biol* 12, r2, 2011.
- McCarthy PT, Cerecedo LR. Vitamin A deficiency in the mouse. *J Nutr* 46, 361–376, 1952.
- McLaren A. Meiosis and differentiation of mouse germ cells. *Symposia of the Society for Experimental Biology* 38, 7–23, 1984.
- Minegishi T, Hirakawa T, Kishi H, Abe K, Ibuki Y, Miyamoto K. Retinoic acid (RA) represses follicle stimulating hormone (FSH)-induced luteinizing hormone (LH) receptor in rat granulosa cells. *Arch Biochem Biophys* 373, 203–210, 2000.
- Morita Y, Tilly JL. Segregation of retinoic acid effects on fetal ovarian germ cell mitosis versus apoptosis by requirement for new macromolecular synthesis. *Endocrinology* 140, 2696–2703, 1999.
- Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, Itoh N, Watanabe Y, Utoguchi N, Tanaka K. Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells. *J Clin Endocrinol Metab* 87, 2830–2837, 2002.
- Nakanishi T, Nishikawa J, Hiromori Y, Yokoyama H, Koyanagi M, Takasuga S, Ishizaki J, Watanabe M, Isa S, Utoguchi N, Itoh N, Kohno Y, Nishihara T, Tanaka K. Trialkyltin compounds bind retinoid X receptor to alter human placental endocrine functions. *Mol Endocrinol* 19, 2502–2516, 2005.
- Nakanishi T, Hiromori Y, Yokoyama H, Koyanagi M, Itoh N, Nishikawa J, Tanaka K. Organotin compounds enhance 17 $\beta$ -hydroxysteroid dehydrogenase type I activity in human choriocarcinoma JAr cells: potential promotion of 17 $\beta$ -estradiol biosynthesis in human placenta. *Biochem Pharmacol* 71, 1349–1357, 2006.
- Nakanishi T. Endocrine disruption induced by organotin compounds; organotins function as a powerful agonist for nuclear receptors rather than an aromatase inhibitor. *J Toxicol Sci* 33, 269–276, 2008.
- Nourashrafeddin S. Potential roles of gonadotropins to control pulsatile retinoic acid signaling during spermatogenesis. *Med Hypotheses* 85, 303–304, 2015.
- Ogata R, Omura M, Shimasaki Y, Kubo K, Oshima Y, Aou S, Inoue N. Two-generation reproductive toxicity study of tributyltin chloride in female rats. *J Toxicol Environ Health A* 63, 127–144, 2001.
- Ohno S, Nakajima Y, Nakajin S. Triphenyltin and tributyltin inhibit pig testicular 17beta-hydroxysteroid dehydrogenase activity and suppress testicular testosterone biosynthesis. *Steroids* 70, 645–651, 2005.
- Omura M, Ogata R, Kubo K, Shimasaki Y, Aou S, Oshima Y, Tanaka A, Hirata M, Makita Y, Inoue N. Two-generation reproductive toxicity study of tributyltin chloride in male rats. *Toxicol Sci* 64, 224–232, 2001.
- Oulad-Abdelghani M, Bouillet P, Decimo D, Gansmuller A, Heyberger S, Dolle P, Bronner S, Lutz Y, Chambon P. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by *stra8*, a novel retinoic acid-responsive gene. *J Cell Biol* 135, 469–477, 1996.
- Piprek RP, Pecio A, Laskowska-Kaszub K, Kloc M, Kubiak JZ, Szymura JM. Retinoic acid homeostasis regulates meiotic entry in developing anuran gonads and in Bidder's organ through Raldh2 and Cyp26b1 proteins. *Mech Dev* 130, 613–27, 2013.
- Richards JS. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev* 60, 51–89, 1980.
- Saitoh M, Yanase T, Morinaga H, Tanabe M, Mu YM, Nishi Y, Nomura M, Okabe T, Goto K, Takayanagi R, Nawata H. Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. *Biochem Biophys Res Commun* 289, 198–204, 2001.
- Saitou M, Yamaji M. Primordial germ cells in mice. *Cold Spring Harbor Perspectives in Biology* 4, 2012.
- Santos NC, Kim KH. Activity of retinoic acid receptor-alpha is directly regulated at its protein kinase A sites in response to follicle-stimulating hormone signaling. *Endocrinology* 151, 2361–2372, 2010.

- Schupp M, Curtin JC, Kim RJ, Billin AN, Lazar MA. A widely used retinoic acid receptor antagonist induces peroxisome proliferator-activated receptor-gamma activity. *Mol Pharmacol* 71, 1251–1257, 2007.
- Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the  $3\beta$ -hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocr Rev* 26, 525–582, 2005.
- Smith CA, Roeszler KN, Bowles J, Koopman P, Sinclair AH. Onset of meiosis in the chicken embryo; evidence of a role for retinoic acid. *BMC Dev Biol* 8, 85, 2008.
- Stel J, Legler J. The Role of Epigenetics in the Latent Effects of Early Life Exposure to Obesogenic Endocrine Disrupting Chemicals. *Endocrinology* 156, 3466–3472, 2015.
- Sylvester SR, Griswold MD. The testicular iron shuttle: a “nurse” function of the Sertoli cells. *J Androl* 15, 381–385, 1994.
- Tabassum S, Pettinary C. Chemical and biotechnological developments in organotin cancer chemotherapy. *J Organomet Chem* 691, 1761–1766, 2006.
- Tan H, Wang JJ, Cheng SF, Ge W, Sun YC, Sun XF, Sun R, Li L, Li B, Shen W. Retinoic acid promotes the proliferation of primordial germ cell-like cells differentiated from mouse skin-derived stem cells in vitro. *Theriogenology* 85, 408–418, 2016.
- Tarrade A, Schoonjans K, Guibourdenche J, Bidart JM, Vidaud M, Auwerx J, Rochette-Egly C, Evain-Brion D. PPAR gamma/RXR alpha heterodimers are involved in human CG beta synthesis and human trophoblast differentiation. *Endocrinology* 142, 4504–4514, 2001.
- Tatone C, Benedetti E, Vitti M, Di Emidio G, Ciriminna R, Vento ME, Cela V, Borzi P, Carta G, Lispi M, Cimini AM, Artini PG. Modulating Intrafollicular Hormonal Milieu in Controlled Ovarian Stimulation: Insights From PPAR Expression in Human Granulosa Cells. *J Cell Physiol* 231, 908–914, 2016.
- Toporova L, Macejova D, Brtko J. Radioligand binding assay for accurate determination of nuclear retinoid X receptors: A case of triorganotin endocrine disrupting ligands. *Toxicol Lett* 254, 32–36, 2016.
- Vergouwen RP, Jacobs SG, Huiskamp R, Davids JA, de Rooij DG. Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. *J Reprod Fertil* 93, 233–243, 1991.
- Walker WH. Molecular mechanisms controlling Sertoli cell proliferation and differentiation. *Endocrinology* 144, 3719–3721, 2003.
- Zhang J, Zuo Z, He C, Wu D, Chen Y, Wang C. Inhibition of thyroidal status related to depression of testicular development in *Sebastiscus marmoratus* exposed to tributyltin. *Aquat Toxicol* 94, 62–67, 2009.
- Zheng WL, Bucco RA, Sierra Rievera E, Osteen KG, Melner MH, Ong DE. Synthesis of retinoic acid by rat ovarian cells that express cellular retinoic acid-binding protein-II. *Biol Reprod* 60, 110–114, 1999.
- Zhuang Y H, Ylikomi T, Lindfors M, Piippo S, Tuohimaa P. Immunolocalization of retinoic acid receptors in rat, mouse and human ovary and uterus. *J Steroid Biochem Mol Biol* 48, 61–68, 1994.
- Yu WJ, Lee BJ, Nam SY, Kim YC, Lee YS, Yun YW. Spermatogenetic disorders in adult rats exposed to tributyltin chloride during puberty. *J Vet Med Sci* 65, 1331–1335, 2003.
- Yu M, Guan K, Zhang C. The promoting effect of retinoic acid on proliferation of chicken primordial germ cells by increased expression of cadherin and catenins. *Amino Acids* 40, 933–941, 2011.
- Yu M, Ge C, Zeng W, Mi Y, Zhang C. Retinoic acid promotes proliferation of chicken primordial germ cells via activation of PI3K/Akt-mediated NF- $\kappa$ B signalling cascade. *Cell Biol Int* 36, 705–712, 2012.