# FLUORIDE TOXICITY IN THE MALE REPRODUCTIVE SYSTEM

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**SUMMARY: This review covers the current scientific understanding of the links between environmental exposure to fluoride (F) and its known or potential effects on human male fertility. The most important consequences of these F exposures are: changes in the structure and functional behavior of spermatozoa, disruption of spermatogenesis, and disturbances of multiple hormone systems that impact male reproduction. The changes in spermatozoa result from oxidative damage, zinc deficiency, and disturbed signal transduction. There is evidence that F interferes with spermatogenesis by depressing levels of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR), modifying G-protein signaling, diminishing levels of testosterone and its androgen receptor (AR), and disturbing levels of estradiol. Furthermore, F is also known to interfere with thyroid hormone metabolism, which directly and indirectly impacts not only spermatogenesis but also other reproductive functions. Although F appears to exert its toxic effects in the male reproductive system through these pathways, the molecular details are still poorly understood. The growing evidence that F overexposure leads to male reproductive toxicity through multiple pathways indicates that an assessment of chronic F exposures in human and animal populations is urgently required.**

Keywords: Dysfunction of spermatozoa; Fluoride reproductive toxicity; Reproductive hormones; Spermatogenesis; Steroidogenesis; Thyroid hormones.

## INTRODUCTION

Although artificial fluoridation of water supplies is practiced in many parts of the world in an effort to reduce the incidence of dental caries, there is growing evidence that the resulting increased exposure to fluoride (F) may cause serious toxic effects.[1] Several clinical investigations and animal experiments suggest that F has adverse impacts on male reproductive function,[2-5] including structural and functional defects in spermatozoa,[6-8] a decrease in sperm count,[4,7,9-11] disturbances in the levels of reproductive hormones,[9,12] alterations in the epididymis and accessory reproductive glands,[13,14] and reduced fertility.[7,15-19] Spermatogonia undergo various processes to ultimately fertilize an oocyte, including spermatogenesis, capacitation, and the acrosome reaction. F has been shown to impair all three of these processes.

In this review, the principal mechanisms discovered so far are considered in the broad categories of impairing the structure and functional behavior of spermatozoa, disturbing the process of spermatogenesis, and altering various hormone levels which influence the male reproductive system.

## FUNCTION OF SPERMATOZOA

Spermatozoa are known to be particularly susceptible to toxic agents.[20] F has been shown to cause a wide variety of structural and functional defects in flagella,

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acrosomes, mitochondria, and nuclei of both spermatids and epididymal spermatozoa[6] (Entry 1 in the Table below for a study on rabbits), rendering spermatozoa nonfunctional.

**Table.** Influence of F on male reproductive system in fluorotic humans and F-intoxicated animals

| No. | Species | Dose | Length of exposure | Effect | Reference |
|---|---|---|---|---|---|
| 1 | Rabbit | 10 mg NaF/kg bw/day | 18 mo | Structural defects in various organelles in spermatozoa, e.g., flagella, acrosome, and mitochondria | 6 |
| 2 | Rat | 4.5 ppm or 9 ppm NaF DW[a] | 75 Days | Decrease in sperm motility and steroidogenic enzymes | 4 |
| 3 | Mouse | 10 mg NaF/kg bw/day | ? | A significant decline in sperm acrosomal acrosin and hyaluronidase | 21 |
| 4 | Rat | 5 mg F/kg bw/day | 8 Weeks | Altered plasma membrane, and decreased ability to undergo acrosome reaction and oocyte fertilization | 22 |
| 5 | Rat | 10 mg NaF/kg bw/day | 30 or 50 Days | Disturbances in energy metabolism in vas deferens and seminal vesicle | 8 |
| 6 | Rat | 5 mg F/kg bw/day; F in serum: 0.263±0.024 ppm | 8 Weeks | Oxidative stress and loss of mitochondrial transmembrane potential | 25 |
| 7 | Mouse | 50, 100, 200, 300 mg NaF/L DW | 8 Weeks | Reduced antioxidative defense and oxidative stress, in two high dose groups, distinct testis cell apoptosis | 26 |
| 8 | Rat | 5 or 26 mg F/L DW | 12 Weeks | Free radical toxicity in testes | 27 |
| 9 | Rat | 100 or 200 ppm F DW | 16 Weeks | Decreased zinc concentrations in testes | 36 |
| 10 | Bank Vole | 200 mg F/mL DW | 4 mo | Decreased testicular zinc concentration | 37 |
| 11 | Bank Vole | 200 mg/mL F DW plus moderate photo period | 4 mo | Decreased zinc concentration in testes | 38 |
| 12 | Mouse | 10 or 100 ppm F DW | 3 mo | Decreased sperm head tyrosine phosphorylation and actin polymerization, and reduced capacitation | 42 |
| 13 | Mouse | 150 mg NaF/L DW | 7 Weeks | Decreased sperm hyperactivation and Catsper1 gene expression level | 46 |
| 14 | Rabbit | 10 mg NaF/kg bw/day | 18 or 29 mo | In 29-mo group, F crossed the blood-testis barrier; cessation of spermatogenesis. | 49 |
| 15 | Mouse | 1000 ppm NaF DW | 3 mo | Necrosis of seminiferous tubules, lack of maturation and differentiation of spermatocytes, and cessation of spermatogenesis. | 50 |
| 16 | Rabbit | 10 mg NaF/kg bw/day | 23 mo | Fragmentation of spermatozoa in epididymis | 13 |
| 17 | Rat | 20 mg NaF/kg bw/day | 28 Days | Inhibition of spermatogenesis and significant diminution in steroidogenic enzymes (3β-HSD, 17βHSD) | 51 |
| 18 | Rat | 50 mg/50 mL NaF into vas deferens | Single injection | Arrest of spermatogenesis and absence of spermatozoa in ST[b] | 52 |
| 19 | Rat | 150 mg NaF/L DW | 10 Days | Decreased expression of EGF & EGFR in spermatogenic cells and Leydig cells | 53 |

[a]DW: drinking water; [b]ST: seminiferous tubules; [c]T: testosterone; [d]HiF: high F; [e]LF: low F; [f]Duration: from 15th day of pregnancy to 4th or 14th day after parturition.

**Table (continued).** Influence of F on male reproductive system in fluorotic humans and F-intoxicated animals

| No. | Species | Dose | Length of exposure | Effect | Reference |
|---|---|---|---|---|---|
| 20 | Rat | 4.5 mg NaF/kg bw/day | 60 Days | Decreased diameter of ST | 19 |
| 21 | Rat | 150 mg NaF/L DW | 50, 100 or 120 Days | Decrease in the diameter of ST and the number of seminiferous epithelial cell layers | 59 |
| 22 | Mouse | 10 or 20 mg NaF/kg bw/day | 30 Days | Severe disorganization and denudation of germinal epithelial cells in ST | 11 |
| 23 | Human | 3-27 mg F/day bw/day or 2-13 mg F/day bw/day | Long Time | A significant reduction in T[c] and increase in FSH | 12 |
| 24 | Human | Fluorosis | Long Time | Decrease in T, increase in FSH and LH | 66 |
| 25 | Rat | Subcutaneous injection of NaF solution | 28 or 38 Days | Decrease in serum estradiol level and apoptosis of spermatogenic cells | 67 |
| 26 | Human | 1.52-6.95 mg NaF/L DW; F in serum: 0.216±0.060 ppm | Long Time | Altered conversion of testosterone into potent metabolite | 68 |
| 27 | Rat | 0.1 or 1.0 mg F daily | 2 mo | Decrease in T4 and T3 level in plasma | 69 |
| 28 | Human | 122±5 μmol/L or 52±5 μmol/L F DW | Long Time | Elevated TSH, decrease in T3 | 70 |
| 29 | Rat | 30 mg F/L DW | ? | Decrease in T3,T4, and thyroid peroxidase | 71 |
| 30 | Rat | 150 mg F/L DW | 120 Days | Decrease in T3 andT4; flattened follicular epithelial cells | 72 |
| 31 | Rat | 20 mg NaF/kg bw/day | 29 Days | Oxidative stress; decrease in T and steroidogenic enzymes | 9 |
| 32 | Human | Skeletal fluorosis | Long Time | A significant reduction in T | 82 |
| 33 | Mouse | 200 or 300 mg NaF/L DW | 8 Weeks | Decrease in AR expression | 84 |
| 34 | Rat | 10 mg NaF/kg bw/day | 50 Days | Significant change in diameter of Leydig cells, reduced steroidogenic enzymes, and disturbance in steroidogenesis | 85 |
| 35 | Rabbit | 4.5 mg/kg bw/day | ? | Degenerative changes in Leydig cells | 86 |
| 36 | Rat | 30 or 100 mg F/L DW | 8 Weeks | Disturbed hormone levels of each layer of the hypothalamus-hypophysis-testis axis | 88 |
| 37 | Human | F in pineal gland: 297±257 mg F/kg | Long Time | Accumulation of F in pineal gland | 89 |
| 38 | Gerbil | HiF[d]: 37 mg F/kg bw/day in food. LF[e]: 7 mg F/kg bw/day in food | 7, 9, 11.5, 16 Weeks | Depressed pineal melatonin output | 3 |
| 39 | Human | 1.52-6.95 mg NaF/L DW; F in serum:0.216±0.060 ppm | Long Time | Fluorosis, significant increase in serum catecholamines, and stimulatory effect on sympathetic nervous system. | 5 |
| 40 | Chick | 500, 1000, 1500, 2000 mg F/kg in food | 150 Days | Karyopyknosis, decreased microvilli and swollen vacuoles in epithelial follicular cells | 110 |
| 41 | Mouse | 500 ppm NaF DW to mother mice | Duration[f] | For the suckling pups: decreased colloid volume | 111 |
| 42 | Pig | 100, 250 or 400 mg F/kg in food | 50 Days | Decrease in activities of Na/K-ATPase and thyroid peroxidase (TPO) | 114 |

[a]DW: drinking water; [b]ST: seminiferous tubules; [c]T: testosterone; [d]HiF: high F; [e]LF: low F; [f]Duration: from 15th day of pregnancy to 4th or 14th day after parturition.

Before discussing the detailed mechanisms by which F is believed to exert its toxic effects, we show in Figure 1 various dysfunctions of spermatozoa caused by F.

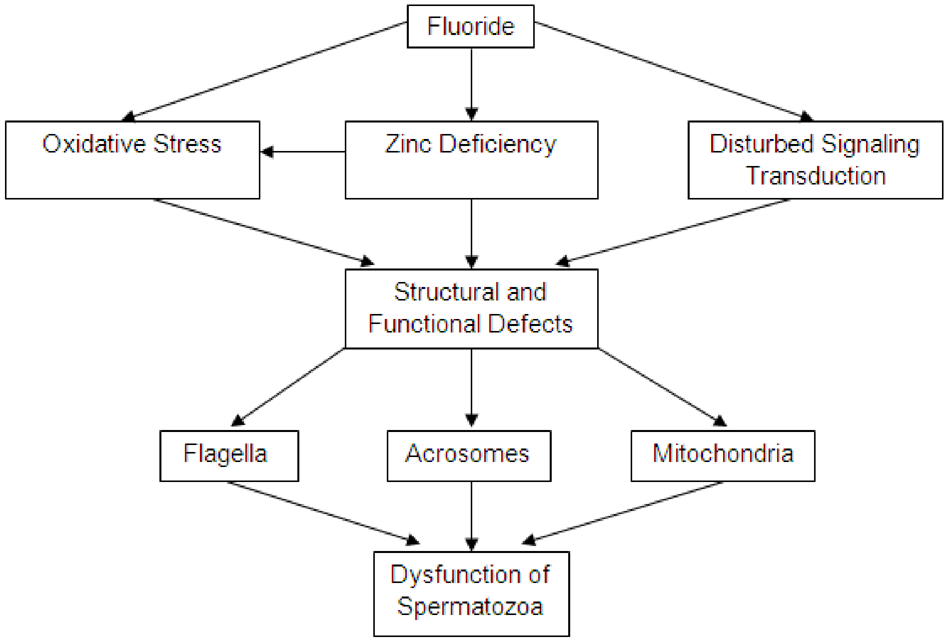


**Figure 1.** F causes dysfunction of spermatozoa by three mechanisms, i.e., oxidative stress, zinc deficiency, and disturbed signaling transduction.

Of particular importance are structural defects in flagella, including their complete detachment from the head of the sperm. Since the flagellum is an organ that imparts motility to spermatozoa, it is conceivable that these changes would result in a decrease in the motility of spermatozoa[4] (Table entry 2 for a study on rats), thereby reducing their capacity to fertilize oocytes.

The acrosome plays an essential role in the process of fertilization, by breaking down the zona pellucida of an ovum in order to facilitate the fusion of two gametes. F-induced structural defects in the acrosome[6] (Table entry 1 for a study on rabbits), and reduced levels of acrosomal acrosin and hyaluronidase[21] (Table entry 3 for a study on mice), may decrease the ability of spermatozoa to carry out the acrosome reaction and to fertilize an oocyte[22] (Table entry 4 for a study on rats).

Spermatozoa need large amounts of energy to fertilize the oocyte. F induces energy deprivation[8] (Table entry 5 for a study on rats) by causing both structural defects[6] (Table entry 1) and inhibition of energy-producing enzymes in mitochondria. Interference of these mitochondrial enzymes occurs both directly, as with glycolytic and Kreb's cycle enzymes,[23] and indirectly, as with peroxynitrite acting on mitochondrial enzymes.[24] Furthermore, F has been shown to cause a loss of mitochondrial transmembrane potential in spermatozoa[25] (Table entry 6 for a study on rats), which enhances its ability to induce energy deprivation. Such F-induced energy deprivation may lead to dysfunction of spermatozoa.

These various defects created by F are attributed to its potential to cause oxidative damage to spermatozoa, to induce zinc deficiency in testes, and to disturb signaling in spermatozoa.

Several studies have shown that chronic F exposure causes oxidative stress[25-27] (Table entries 6–8). Spermatozoa are particularly vulnerable to lipid peroxidation because their plasma membranes contain large quantities of polyunsaturated fatty acids.[28,29] The cytoplasms of spermatozoa also contain low concentrations of reactive oxygen species (ROS) scavenging enzymes which may not be sufficient to neutralize the increased ROS production due to oxidative stress.[30] F exposure may induce lipid peroxidation, leaving the intracellular antioxidant enzymes unable to protect the plasma membrane that surrounds the mitochondria, acrosome and the tail,[31,32] from defects. The resulting loss of plasma membrane fluidity and integrity may also interfere with sperm-oocyte fusion events.[33] An increase in oxidative stress may also lead to DNA damage,[34] which would increase the frequency of abnormal sperm.[35]

High F intake can lead to zinc deficiency in testes and the male reproductive system[36-38] (Table entries 9–11). Zinc deficiency may suppress the testosterone levels critically necessary for testis development,[39] and, more importantly, it increases oxidative stress in testes leading to poorer quality spermatozoa.[39-41]

Finally, F has also been shown to disturb signaling systems, especially those involving capacitation and acrosome reactions of spermatozoa[42] (Table entry 12 for a study on mice). Tyrosine phosphorylation may be the primary or even the exclusive indicator of the signal transduction needed to capacitate sperm,[43,44] and actin polymerization is one of the crucial aspects enabling sperm to undergo the acrosomal reaction.[45] F has been shown to decrease sperm head tyrosine phosphorylation and actin polymerization[42] (Table entry 12), thereby decreasing capacitation and acrosome reactions in spermatozoa[22,42] (Table entries 4 and 12).

It has been suggested that F disturbs the calcium signaling pathway involved in sperm hyperactivation.[46] Sperm hyperactivation is a type of sperm motility that is necessary for successful penetration of the zona pellucida. Given that Catsper1 protein is involved in the calcium signaling pathway during the hyperactivation process,[47,48] F could interfere with the calcium signaling pathway during the hyperactivation process by reducing Catsper1 gene expression[46] (Table entry 13 for study on mice). In turn, disturbance in the calcium signaling pathway could be expected to impair sperm hyperactivation, resulting in dysfunction of spermatozoa.

## SPERMATOGENESIS

A blood-testis barrier protects spermatogenic cells and the process of spermatogenesis. It has been demonstrated that F may cross this permeable barrier during prolonged exposure[49] (Table entry 14 for a study on rabbits), perhaps by causing necrosis of seminiferous tubules in testes[50] (Table entry 15 for a study on mice). Once it has crossed the barrier, F causes a lack of maturation and differentiation of spermatocytes, fragmentation of spermatozoa in the epididymis, and even cessation of spermatogenesis.[13,49,50] (Table entries 14–16) Therefore, F can impair the process of spermatogenesis[51] (Table entry 17 for a study on rats), as shown indirectly in a study by Chinoy et al.[52] (Table entry 18 for a study on rats), in which arrest of spermatogenesis and absence of spermatozoa in the seminiferous tubules were observed when F was injected into the vas deferens of adult male albino rats.

After crossing the barrier, F impairs the process of spermatogenesis mainly through the following five mechanisms shown in Figure 2: reducing the expression of epidermal growth factor (EGF) and its receptor (EGFR) in spermatogenic cells; modifying G-protein signaling in both Leydig cells and Sertoli cells; diminishing levels of testosterone and its receptor (AR); disturbing levels of estradiol; interfering with thyroid function.

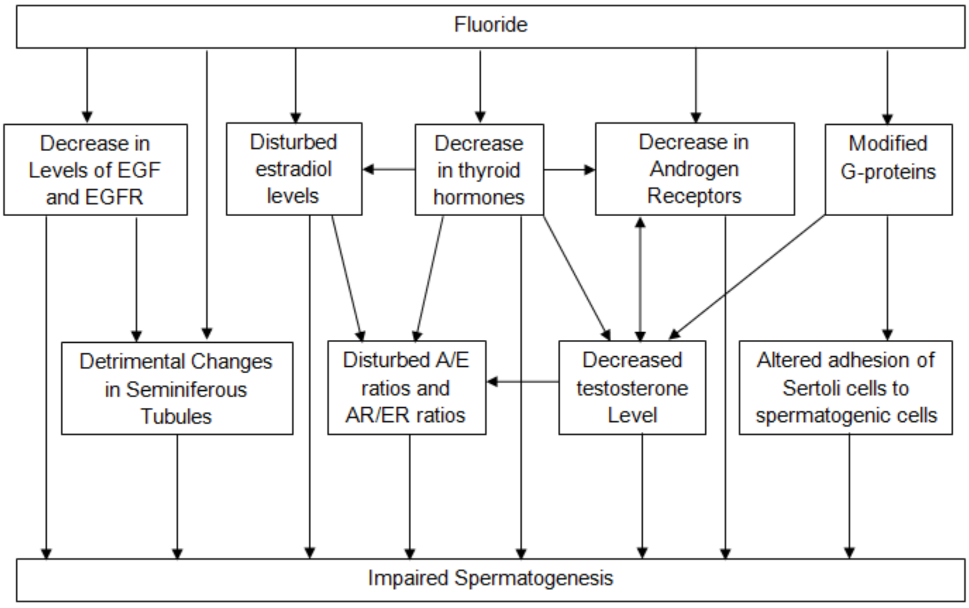


**Figure 2.** F interferes with spermatogenesis through five mechanisms.

Research by Wan et al.[53] (Table entry 19 for a study on rats) revealed that F caused decreased expression of EGF and EGFR in spermatogenic cells. These changes are significant since EGF and EGFR not only mediate normal spermatogenic proliferation,[54] but also act as irreplaceable mediators of spermatogenesis.[55-58] Moreover, F has been shown to promote the following effects: decrease the diameter of seminiferous tubules[19,59] (Table entries 20, 21); produce significant disorganization and denudation of germinal epithelial cells of seminiferous tubules[11] (Table entry 22 for study on mice); diminish the number of seminiferous epithelium cell layers[59] (Table entry 21 for study on rats).

Compromising the seminiferous epithelium may result in complete cessation of spermatogenesis under severe conditions. Conceivably, F causes these changes in the seminiferous tubules by reducing expression of EGF and EGFR, since EGF and EGFR promote proliferation of seminiferous epithelium cells. However, this remains speculative, since we are unaware of research examining this question. Nevertheless, one can assume at the very least that F can lead to reduction or cessation of spermatogenesis by reducing the level of EGF and EGFR and causing degenerative changes in seminiferous tubules.

In addition, F interferes with spermatogenesis by modifying important cell signal transducers called G-protein coupled receptors[60] which are used by the pituitary neurohormone called luteinizing hormone (LH).[61] LH is an important regulator of testosterone production in Leydig cells. Therefore, F-induced modification of G-proteins could inhibit the release of testosterone, and, since testosterone is essential for the initiation of spermatogenesis, this inhibition would eventually lead to low levels of testosterone, thereby impairing spermatogenesis[62,63]. Moreover, Sertoli cell adhesion to spermatogenic cells is dependent on G-protein signaling.[64] Since Sertoli cells play an important role in spermatogenesis,[65] alterations of Sertoli cell adhesion caused by modified G-proteins can be expected to interfere with spermatogenesis.

F has also been shown to decrease levels of testosterone and AR, disturb the level of estradiol, and interfere with thyroid hormones. Since these effects disturb not only spermatogenesis but also other reproductive functions, they are considered in the next section wherein are examined how these effects interfere with spermatogenesis and other reproductive functions.

## HORMONE LEVELS

F has been shown to disrupt various hormones involved in male reproduction. The available research indicates that F exposure is clearly associated with the following: increased levels of follicle stimulating hormone (FSH) and LH[12,66] (Table entries 23 and 24); decreased estrogen levels[67] (Table entry 25 for study on rats); decreased testosterone levels and changes in its conversion into its potent metabolites[12,66,68] (Table entries 23, 24, and 26); reduced thyroid hormones[69-72] (Table entries 27–30); disturbed androgen to estrogen ratios (A/E)[73-77] and estrogen receptor to androgen receptor ratios (ER/AR).[78-81] Such disturbances in multiple endocrine axes would probably contribute to male reproductive disorders.

### *Testosterone*

Several studies have revealed that F can lead to a decrease in testosterone[9,12,66,82] (Table entries 23, 24, 31, and 32), which is essential for the initiation of spermatogenesis.[62,63] Before discussing the mechanisms by which diminished testosterone and AR disturb spermatogenesis, we will first review the main mechanisms through which F is thought to decrease testosterone levels: inducing changes in both structures and enzyme activities in Leydig cells, and interfering with hypothalamus-hypophysis-testis axis.

Leydig cells require normal expression and function of EGFR, AR, and G-proteins[83] in order to synthesize testosterone. F exposure has been shown to reduce EGFR and AR expression[53,84] (Table entries 19 and 33), and to interfere with G-proteins in Leydig cells,[60] potentially impacting both the normal function and normal level of testosterone. F has also been shown to cause both a significant change in the diameter of Leydig cells[85] (Table entry 34 for study on rats) and extensive degenerative alterations in them[86] (Table entry 35 for study on rabbits). Such structural changes would decrease the ability of Leydig cells to synthesize testosterone. However, the most important mechanism by which F reduces the level of testosterone is interference with steroidogenesis in Leydig cells[85] (Table entry 34). This interference has been demonstrated in several studies in which activity levels of testicular steroidogenic marker enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) decreased significantly in NaF-treated rats[4,9,51] (Table entries 2, 17, and 31). Since testicular steroidogenesis is controlled by these two rate-limiting enzymes, a decline in their activities in Leydig cells significantly decreases the production and therefore the level of testosterone. This is supported by a study where hypotestosteronemia in AR-depleted was not caused by reducing the number of Leydig cells, but instead, by alterations of several key steroidogenic enzymes, including 3β-HSD and 17β-HSD.[87]

Moreover, F has been found to interfere with the hypothalamus-hypophysis-testis axis[88] (Table entry 36 for study on rats) by modulating melatonin and catecholamine levels.

F is known to accumulate in the pineal gland[89] (Table entry 37 for study on humans) and to inhibit the release of melatonin by the pineal gland[3] (Table entry 38 for study on gerbils). Since melatonin has an anti-gonadotropic effect,[90-92] it is conceivable that F inhibition of melatonin indirectly but significantly increases the level of gonadotropins [12,66] (Table entries 23 and 24). Under normal circumstances, elevated levels of gonadotropic hormones would result in increased testosterone levels. If there is an inability to increase testosterone due to F interference, as described in this review, elevated gonadotropic hormones may be sustained, without eliciting a compensatory elevation of testosterone. For Sertoli cells, the proliferative signals from gonadotropins seem to be balanced by the antiproliferative signals from the genomically active thyroid hormone, triiodothyronine (T3).[93] Any imbalance in these opposing signals (e.g., decreased T3, increased gonadotropins) may lead to some pathological consequences, e.g., testicular tumors.[94]

In addition, it has been suggested that F interferes with the hypothalamus-hypophysis-testis axis[88] (Table entry 36) by causing an increase in the level of catecholamines[5] (Table entry 39 for study on humans). The ability of F to cause an elevated level of catecholamines is indirectly supported by a study in which F stimulates the release of catecholamines from NaF-cultured (15–30 mM) bovine adrenal chromaffin cells.[95] Since elevated catecholamines would have a stimulatory effect on the sympathetic nervous system, they can be expected to influence the hypothalamus-gonadal axis and cause marked changes in the levels of reproductive hormones.[5]

As explained above, F overexposure decreases the level of testosterone, which is essential for the initiation of spermatogenesis.[62,63] Moreover, a marked reduction in intratesticular testosterone concentrations appears to be an important initiator of germ cell apoptosis in the seminiferous epithelium.[96,97] Therefore, any reduction of testosterone levels by F would be expected to interfere with the initiation of spermatogenesis, and lead to an increase of germ cell apoptosis.

F has also been shown to reduce the expression of AR[84] (Table entry 33 for study on mice). Since the functional ARs in Sertoli cells and Leydig cells play a vital role in spermatogenesis and steroidogenesis,[65,87] respectively, any reduction in the level of AR caused by F, impairs the process of spermatogenesis and decreases the level and function of testosterone. Since androgens stimulate AR expression in Sertoli cells,[63,98,99] the resulting decreased testosterone would lower the level of AR expression, leading to a vicious adverse cycle.

*Estrogens*

As already noted, F has been shown to reduce the level of estradiol significantly[67] (Table entry 25 for study on rats). This effect is important because estrogens also play a critical role in the initial development of the male reproductive axis. Estradiol has been shown to prevent apoptosis of male germ cells.[100] Thus, a F-induced decrease in estradiol levels may facilitate apoptosis of spermatogenic cells, leading to a reduction or cessation of spermatogenesis. Moreover, estradiol stimulates testis development in hypogonadal mice by a direct action on estrogen receptors (ER) and by stimulating the pituitary neurohormone called follicle stimulating hormone (FSH) in a positive feedback loop, during a specific early temporal window in male reproductive development.[101-104] FSH is thus an important signal for increasing spermatogenesis. Although decreased estrogen levels are known to increase FSH in a negative feedback loop, a F-induced decrease in estradiol level during this period may reduce FSH, leading to decreased spermatogenesis. In short, by reducing estradiol concentration, F overexposure induces apoptosis of spermatogenic cells[67] (Table entry 25) and decreases spermatogenesis, thereby causing the arrest of spermatogenesis.

*Thyroid Hormones*

Endocrine systems influence each other like lined-up dominoes. Among them, two important endocrine axes—gonadal and thyroidal—are strictly interdependent.[105] Disturbance in normal levels of thyroid hormones generally results in decreased fertility and sexual activity.[106] Moreover, hypothyroidism, a condition characterized by abnormally low serum thyroid hormones, is associated with a marked delay in sexual maturation and development.[107] Therefore, thyroid hormones deserve special attention in uncovering the toxic effects of F on the male reproductive system. First we consider the ways in which F induces decreased thyroid hormones. Then we examine mechanisms by which decreased thyroid hormone levels impair normal functions of the male reproductive system.

F has been shown to increase thyroid stimulating hormone (TSH) and reduce triiodothyronine (T3) and thyroxine (T4)[69-72] (Table entries 27–30), thereby causing hypothyroidism in some populations.[108,109] F is considered to interfere with thyroid hormone levels mainly through three mechanisms: impairing normal structures of the thyroid gland, disrupting iodine metabolism in thyroid glands, and interfering with tissue-specific metabolism of thyroid hormones.

Several studies reveal that F can directly damage the structures of thyroid follicles, resulting in the following abnormalities: flattened follicular epithelial cells, reduced cytoplasm, karyopyknosis of follicular epithelial cells, decreased microvilli, and swelling of vacuoles.[72,110] (Table entries 30 and 40) Since thyroid follicles are the active sites for synthesizing thyroid hormones, these structural disruptions by F may disrupt the synthesis of thyroid hormones in thyroid glands. Moreover, F has been shown to cause decreased colloid volume[111] (Table entry 41 for a study on mice) and suppress the endocytosis of colloid,[112] resulting in decreased thyroid secretion.

Iodine is essential in the biosynthesis of thyroid hormones.[113] Therefore, any factor that influences the uptake, transport, and metabolism of iodine will affect the normal biosynthesis and utilization of thyroid hormones.

A study by Zhan et al.[114] (Table entry 42 for study on pigs) revealed that F inhibited the activity of Na/K-ATPase. In addition, Clinch[115] in her review pointed out that F interferes with the activity of Na/K-ATPase and the sodium-iodide symporter. Since iodide uptake is facilitated by the combined actions of the Na/K-ATPase and the sodium/iodide symporter,[115] a decrease in the activities of these enzymes caused by F would reduce the uptake of iodide in the thyroid gland and the subsequent production of thyroid hormones. High F intake has also been shown to inhibit the activity of thyroid peroxidase (TPO)[114] (Table entry 42). Since TPO is an enzyme which is essential for the production of thyroid hormones, decreased activity of TPO caused by F would also lead to reduced thyroid hormone synthesis. Clinch[115] has also pointed out that F interferes with the deiodinase enzymes that are required for tissue-specific metabolism of T4.

Thyroid hormone disruption caused by F is thought to interfere with the normal functions of the male reproductive system by the following six mechanisms: disrupting normal development of testes; lowering libido; reducing sex hormones; interfering directly and indirectly with spermatogenesis; influencing steroid hormone receptors; inducing oxidative stress in testes.

It is clear that thyroid hormones are critical to testicular development.[116] Several studies have revealed that T3 stimulates Sertoli cell differentiation[117] and modulates Sertoli cell maturation[118] by inhibiting the activity of aromatase in Sertoli cells.[73-77] Aromatase is considered to be a Sertoli cell functional maturation marker[74] because this enzyme modulates the critical A/E hormone ratios by controlling the conversion of androgens into estrogens.[73] For example, increased aromatase enhances the conversion into estrogens and decreases the pool of androgens, whereas inhibition of aromatase increases androgen levels, while decreasing estrogen levels. Therefore, a F-induced decrease in thyroid hormones, especially T3, may result in increased aromatase activity, a decrease in the level of androgens, and an increase in the level of estrogens. Since androgens play an irreplaceable role in triggering differentiation of Sertoli cells[119] whereas estrogens have a negative effect on Sertoli cells differentiation and development,[120] such a decrease in the A/E hormone ratios would be expected to result in underdevelopment of testes.

Hypothyroidism is known to be associated with impotence and decreased libido,[121] since thyroid hormones affect brain chemistry involved in sexual arousal, which in turn stimulates the autonomic nervous system and affects many other hormones necessary for energy (e.g., cortisol) and sexual stimulation (e.g., oxytocin, estradiol, and the androgen family).[122]

People with hypothyroidism have been found to present a low serum testosterone concentration,[123-125] which returned to normal after receiving thyroid hormone supplementation.[124] Furthermore, several studies have indicated that people with hypothyroidism had low levels of dehydroepiandrosterone (DHEA),[126,127] which is a prohormone for sex steroids. Hypothyroidism induces decreased serum testosterone concentration by acting on Leydig cells, which are the active sites for synthesizing most of the androgens. This effect has been demonstrated by several studies in which Leydig cells from hypothyroid adult rats harbor low activities of 3β-HSD and 17β-HSD under both basal and LH-induced conditions,[128] and produce less testosterone than from normal rats.[129] The resulting decrease in testosterone levels would interfere with spermatogenesis as described above. Moreover, a recent study by Wajner et al.[130] revealed that type 2 iodothyronine deiodinase, which regulates the tissue-specific conversion of T4 to the genomically active T3, is predominantly expressed in elongated spermatids, suggesting that thyroid hormones might have a direct effect on spermatogenesis.

Hypothyroidism has also been shown to influence sex steroid hormone receptors, which are the active sites for sex hormones to carry out their vital functions. Studies indicate that T3 not only influences the A/E hormone ratios, as described above, but also up-regulates the transcription of AR gene[78] and the expression of AR[79] in Sertoli cells, and down-regulates estrogen receptors (ER).[80,81] This T3-induced increase in the AR/ER ratios promotes spermatogenesis[65] and functional maturation of Sertoli cells.[80] Low levels of AR and high levels of ER in Sertoli cells resulting from F-induced low T3, would presumably decrease spermatogenesis and lead to underdevelopment of testes. Also, since thyroid hormones have been shown to prevent estrogen-induced proteolysis of ER in lactotrope cells of the pituitary,[131] and to be effective in increasing the concentration of cytosolic ER in the pituitary,[132] a decrease in thyroid hormones may cause derangements in the estrogen/ER signaling, known to be important in male reproductive function.[101,133]

Finally, transient and congenital hypothyroidism has been shown to induce oxidative stress in testes by reducing the levels of testicular enzymatic and nonenzymatic defenses.[134,135] The resulting oxidative stress may cause detrimental effects as described above.

In short, F can interfere with the biosynthesis and function of thyroid hormones in the thyroid gland and male reproductive glands, resulting in the disruption of the normal functions of the male reproductive system**.**

## CONCLUSION

Our current scientific understanding of the potential links between environmental exposure to F and decreasing human fertility rates is growing. From overexposure in the environment, F exerts its toxic effects by disturbing normal architecture and functions of spermatozoa, impairing the process of spermatogenesis, and disrupting hormone levels required for male reproduction. More research is urgently needed to elucidate the molecular pathways involved, and to determine how low the F intake levels and F blood levels from chronic exposure must be in order to cause no harm.

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