

## *trans*-Resveratrol, a Natural Antioxidant from Grapes, Increases Sperm Output in Healthy Rats<sup>1</sup>

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**ABSTRACT** *trans*-Resveratrol was reported to have health benefits including anticarcinogenic effects and protection against cardiovascular disease. One of the mechanisms by which it exerts its action is through modulating the estrogen response systems. Because estrogen is involved in male reproductive biology, we investigated the effect of *trans*-resveratrol on testis and spermatogenesis. Adult male rats were divided into 2 groups. The treated group was administered by gavage 20 mg/(kg · d) of *trans*-resveratrol suspended in 10 g/L of carboxymethylcellulose for 90 d, whereas the control group received only carboxymethylcellulose during the same period. The relative weight of testes did not differ between the groups. However, the diameter of the seminiferous tubules was significantly reduced from 437.5 ± 0.1 μm in the controls to 310.9 ± 0.1 μm in the resveratrol-treated rats. This decrease was accompanied by a significant increase in tubular density, from 3.20 ± 0.18 in controls to 6.58 ± 0.18 tubules/mm<sup>2</sup> in the treated group. Moreover, sperm counts were significantly greater in the resveratrol-treated rats (24.8 ± 3.30 × 10<sup>7</sup>) than in the control group (14.1 ± 0.80 × 10<sup>7</sup>), but sperm quality did not differ. Serum concentrations of gonadotrophins and testosterone were significantly higher in the resveratrol-treated group. We identified a novel activity of *trans*-resveratrol. The daily oral administration of this phytochemical to adult male rats enhanced sperm production by stimulating the hypothalamic-pituitary-gonadal axis, without inducing adverse effects. J. Nutr. 135: 757–760, 2005.

**KEY WORDS:** • *trans*-resveratrol • phytochemical • spermatogenesis • testis • rats

*trans*-Resveratrol (*trans*-3,4',5-trihydroxystilbene) is a natural antioxidant that is widely consumed in the Mediterranean Diet in the form of peanuts, grapes, and wine. The interest in compounds present in wine increased when epidemiologic studies indicated an inverse correlation between red wine consumption and the incidence of cardiovascular disease. This finding prompted considerable interest in the possible effects of *trans*-resveratrol, leading to the description of several beneficial effects on health. In addition of being an antioxidant and a vasorelaxing agent, it modulates lipoprotein metabolism, inhibits platelet aggregation, and exerts cancer chemopreventive and therapeutic activity (1,2). In eliciting these actions, *trans*-resveratrol triggers a variety of established cellular and molecular effectors, the most remarkable of which is the estrogen response systems. Given the structural similarities of *trans*-resveratrol to diethylstilbestrol (DES)<sup>3</sup> and estradiol, and

its activity as a modulator of the estrogen-response systems, it has been classified as a phytoestrogen (1,2).

Estrogens, which are traditionally considered female hormones, are also involved in the male reproductive system, which is classically thought to be controlled mainly by androgen hormones and their receptors. Estrogens, derived either from local aromatization of androgens or produced by the testes, can exert feedback action on the neuroendocrine components of the male reproductive axis. They also have paracrine actions within the testes (3–5). *trans*-Resveratrol modulates the estrogen-response systems and may therefore be involved in male reproduction. Consequently, the aim of the present study was to investigate the effect of a 90-d *trans*-resveratrol treatment [20 mg/(kg · d)] on testes and spermatogenesis of adult rats. Because spermatogenesis involves a complex interplay between the structural elements of the testes and the endocrine system, the serum concentrations of the reproductive hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured.

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<sup>3</sup> Abbreviations used: AR, androgen receptor; DES, diethylstilbestrol; ER,

estrogen receptor; FSH, follicle stimulating hormone; LH, luteinizing hormone; ROS, reactive oxygen species.

## MATERIALS AND METHODS

**Chemicals and reagents.** *trans*-Resveratrol (Sigma, Tres Cantos) was chemically pure. Before use, its purity was assessed by HPLC coupled to a diode-array UV detector; a chromatogram that showed a single peak at 306 nm, its maximum absorbance, was obtained. The use of this detector allowed confirmation of the identity of the peak by its spectrum (data not shown). Dose preparation, administration, and sample treatments were performed in dim light to avoid photochemical isomerization of *trans*-resveratrol to the *cis* form. All other reagents were commercially available, analytical-grade chemicals.

**Animals.** Male Sprague-Dawley rats, weighting 210–220 g, were purchased from breeding colonies (Harlan Ibérica) and quarantined for 1 wk. They were housed in cages (3 rats/cage) at  $22 \pm 3^\circ\text{C}$ , with 40–70% relative humidity and controlled lighting that provided a 12-h light:dark cycle. Water and a solid diet (Rodent Toxicology Diet, B&K Universal) were freely available.<sup>4</sup> No traces of *trans*-resveratrol were detected in the commercial diet or in the plasma from control rats, as revealed by the analyses performed using the method of Juan et al. (6). Rats were handled and killed following the European Community guidelines for the care and management of laboratory animals. The studies were approved by the Animal Experimentation Research Committee of the University of Barcelona. All rat manipulations were performed in the morning to minimize the effects of circadian rhythm.

**Experimental design and dose preparation.** Rats were randomly divided into 2 groups, a resveratrol group ( $n = 12$ ) and a control group ( $n = 12$ ). The resveratrol group was orally administered, by gavage, 20 mg/kg of *trans*-resveratrol at a constant volume of 10 mL/kg body weight, every day for 90 d. Because of its low solubility in water, *trans*-resveratrol was suspended in 10 g/L carboxymethylcellulose. The dose was adjusted according to the rat's weight to ensure a constant dose level and was freshly prepared immediately before each administration. The control group was administered only carboxymethylcellulose during the same period. The dosage selected corresponded to 1000 times the amount consumed by a 70-kg person who drinks 250 mL of red wine a day containing 1.4 mg of *trans*-resveratrol, a dose that is not harmful to rats (7). A period of 90 d was chosen to cover the complete spermatogenesis cycle of rats.

Skin, eyes, mucous membranes, respiratory system, autonomic and central nervous system conditions, somatomotor pattern, and behavior were examined daily. Body weight and food and water consumption were recorded daily. The growth rate was calculated as the difference between the final weight and the initial weight divided by 90 d.

At the end of the study, rats were food deprived overnight and anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg). Blood samples were collected by cardiac puncture; 2 mL was transferred to a tube without anticoagulant for hormone analysis and 1 mL was placed in EDTA for *trans*-resveratrol determination. Whole blood was centrifuged at  $1500 \times g$  (model TJ-6 centrifuge, rotor TH-4 with buckets, Beckman Coulter) for 15 min at room temperature.

**Plasma *trans*-resveratrol and its conjugates.** At 24 h after the last oral administration, the *trans*-resveratrol concentration was measured using the method described by Juan et al. (6), which allows the determination of the free compound as well as its sulfate and glucuronide conjugates in plasma samples.

**Testicular morphometry.** Testes, epididymae, and ducti deferens were carefully dissected and the testes wet weight was recorded. Results were expressed as testes weight relative to 100 g of body weight. Subsequently, testes were fixed in 1.23 mol/L buffered formaldehyde pH 7.4 (Sigma Diagnostics) and kept at  $4^\circ\text{C}$  until analysis. Testes were dehydrated in an alcohol gradient and placed in xylene. They were then embedded in paraffin and cut into small sections (1 mm<sup>3</sup>). The tissue blocks were oriented so that the seminiferous tubules could be sectioned transversely into 5- $\mu\text{m}$  slices, thereby giving round or rounded tubules, which were stained using the hematoxylin-eosin technique. Morphometrical measures were performed on 5 distinct areas and a total of 250–300 seminiferous tubules were measured in each rat (8). The diameter of the testicular

tubules was determined by projecting the slides at 50X; measurements were systematically made from a set of 5 regions with presized areas. The smallest minor axis diameter of each of the 5 randomly selected sections of each rat was measured. Images were taken in a Nikon Eclipse E800 optical microscope (Nikon Europe) linked to a digital Sony 3CCD camera (Sony Inc. Europe). The digitalized images were then processed using the ANALYSIS 2.1 imaging package (Soft-Imaging Systems GmbH).

**Testicular histology.** Formaldehyde-fixed testes were embedded in paraffin and sliced on silane-precoated slides (slice thickness: 3–4  $\mu\text{m}$ ). These slices were further deparaffined with xylol, and histological observations were performed after staining using the hematoxylin-eosin method. For long storage, slides were mounted using a commercial mounting medium (Adh CLINIC<sup>®</sup>, Clinic Services). The samples were observed under an Olympus microscope (model BX50) coupled to a photographic camera.

**Sperm counts and morphology.** Sperm contained in the epididymae/ducti deferens complex from both testes of each rat were released together into 1 mL of buffered formaldehyde. The sperm count was measured in a hemocytometer chamber. A 10- $\mu\text{L}$  aliquot of sperm suspension was stained using the vital Eosin-Nigrosin staining technique (9). The percentage of sperm morphological abnormalities was calculated in these stained spermatozoa after counting 200–300 in an optical microscope at 1000X augmentations in a bright field. The percentage of abnormalities was calculated, first as a total, and then further classified in relation to the specific location of each abnormality in the sperm cell. Consequently, total abnormalities were classified as head abnormalities, neck and midpiece abnormalities, distal cytoplasmic droplets, and tail abnormalities.

**Hormone assays.** After centrifugation, serum was removed from the clot, immediately frozen in liquid N<sub>2</sub> and stored at  $-80^\circ\text{C}$  until analysis. The serum concentrations of LH, FSH, and testosterone were measured by ELISA. Specific commercial kits for rats were used to quantify FSH (RPN 2560), LH (RPN 2562) (Amersham) and testosterone (EIA-1559, DRG Instruments) concentrations.

**Statistical analysis.** Results were expressed as means  $\pm$  SEM. Means were compared by the Student-Neuman-Keuls Test (STATISTICA 6.0 for Windows software, StatSoft). Differences with  $P < 0.05$  were considered significant.

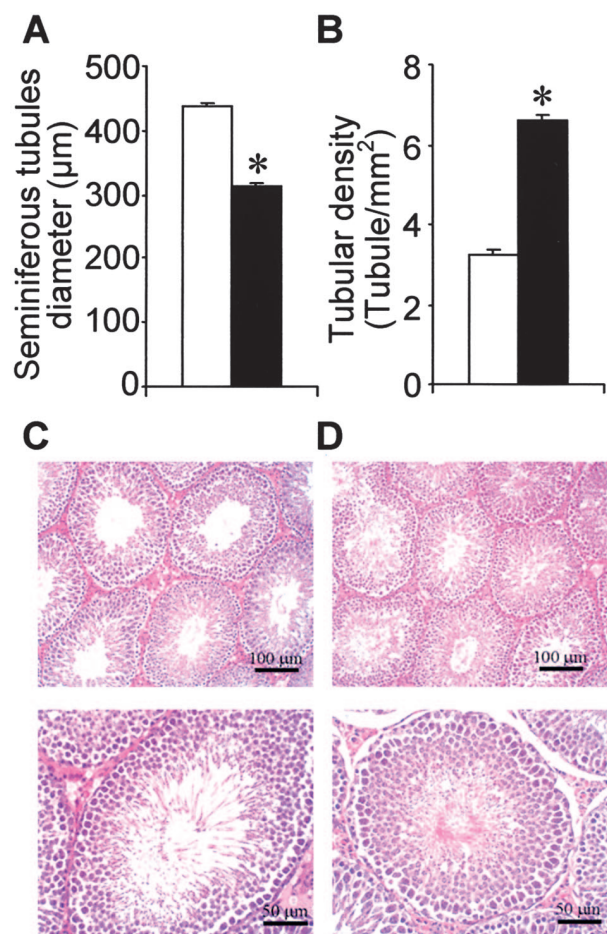
## RESULTS

**Body weight and food and water consumption.** *trans*-Resveratrol did not have adverse effects during the experimental period. The body weight gain of the resveratrol-treated group did not differ from that of the controls; both groups grew steadily throughout the study. Thus, the body weight of the resveratrol group increased from  $219 \pm 2$  g ( $n = 12$ ) on d 1 to  $397 \pm 9$  g on d 90, whereas in the control group, it increased from  $216 \pm 2$  g ( $n = 12$ ) on d 1 to  $394 \pm 6$  g ( $n = 12$ ) on d 90. Furthermore, the consumption of food and water did not differ between the 2 groups.

**Plasma *trans*-resveratrol and its conjugates.** The *trans*-resveratrol plasmatic concentrations determined by HPLC with diode-array detection was  $66.7 \pm 12.0$  nmol/L. Free *trans*-resveratrol was the only compound detected in plasma 24 h after the oral administration in food-deprived rats. No traces of sulfate or glucuronide conjugates were found.

**Testicular histology and morphometry.** *trans*-Resveratrol did not affect testicular gross anatomy or wet weight. The relative weights of the testis in the control and resveratrol groups were  $0.52 \pm 0.02$  and  $0.49 \pm 0.01$ , respectively. Moreover, histological examination did not reveal microscopic lesions such as cytoarchitectural alterations or disorganization of the tubular elements in either group (Fig. 1). However, structural changes in the density of the seminiferous tubules were observed (Fig. 1). In the resveratrol group, the tubes were of reduced diameter but of increased length, which doubled the tubular density ( $P < 0.05$ ).

<sup>4</sup> The commercial diet contained (g/kg) 160.4 crude protein, 26.0 crude fiber, 467.4 carbohydrate, 29.5 lipid, and 42.2 ash.



**FIGURE 1** Seminiferous tubules of control male rats (white bars) and those administered 20 mg/kg of *trans-resveratrol* (black bars) for 90 d. (A) Diameter; (B) tubular density; (C,D) histological appearance of testis sections of control (C) and *trans-resveratrol*-treated rats (D). Values are means + SEM,  $n = 12$ . \*Different from control,  $P < 0.05$ .

**Sperm counts and morphology.** The sperm count was 76% greater in treated rats than in controls ( $P < 0.01$ ) (Table 1). Sperm abnormalities, including misshapen head, middle piece, or principal piece and distal cytoplasmic droplet were not more frequent in resveratrol-treated rats (Table 1). Consequently, the percentage of atypical forms was not affected by the treatment, indicating that the overall sperm quality was not impaired by *trans-resveratrol*.

**Serum hormones.** At the end of the study, serum concentrations of FSH, LH, and testosterone were greater in the resveratrol-treated rats than in the control group (Table 1).

## DISCUSSION

Our study is the first to describe a novel effect of *trans-resveratrol*, namely, an increase in spermatozoa production in healthy rats. Mammalian testes fulfill 2 main functions, the synthesis of steroid hormones and the production of spermatozoa, both of which are controlled by gonadotrophins and testosterone as well as locally produced factors (10). Our results show that these 2 functions were enhanced in male rats by the daily oral administration of *trans-resveratrol*. These changes were not accompanied by side effects related to the compound. This lack of toxicity was not surprising because we previously demonstrated that the oral administration of *trans-*

resveratrol at a dose of 20 mg/(kg · d) for 28 d was not harmful to male rats (7). Thus, the absence of symptoms observed throughout the 90-d treatment, the lack of negative effects on development, and the normal appearance of the vital organs in the gross necropsy indicate that *trans-resveratrol* is nontoxic under these experimental conditions. Moreover, our results are substantiated by a recent toxicological study that reports that the exposure of male and female rats to a dose of 300 mg/(kg · d) of *trans-resveratrol* for 28 d did not have adverse effects (11).

The repeated oral administration of *trans-resveratrol* induced a 71% decrease in the mean diameter of the seminiferous tubules with a 100% increase in the testicular tubular density. Together, these changes led to an overall increase in the size of the spermatogenic tissue. Therefore, this enlargement may explain the increase in sperm production observed. It is noteworthy that sperm collected from the epididymis had matured properly because the morphological examination revealed the same percentage of abnormalities between groups.

This is the first report of a stimulatory effect of *trans-resveratrol* on the secretion of gonadotrophins, the major endocrine regulators of spermatogenesis. The concentrations of FSH, which acts within the tubules to stimulate spermatogenesis, and LH, which signals the production of testosterone in Leydig cells, were elevated in the resveratrol group compared with the control rats. Testosterone, which is essential for promoting spermatogenesis, was also enhanced. These results indicate that the effect of *trans-resveratrol* on sperm count may be caused by the hypophysary stimulation of testicular function. The endocrinal regulation of the hypothalamic-pituitary-gonadal axis in the males is intricate and involves estradiol and testosterone (12,13). The sites for the feedback regulation include cells in the hypothalamus, which are in close proximity to gonadotrophin-releasing hormone neurons, and gonadotrophins in the pituitary, which may respond directly to androgens caused by the expression of the androgen receptor (AR), whereas the presence of aromatase and the estrogen receptor (ER) allows the conversion of androgens into estrogens, and the subsequent activation of ER signaling pathways (12). A possible explanation for our findings could be attributed to the binding of *trans-resveratrol* to ER as a mixed weak agonist/antagonist, without estrogenic properties (1,2,14,15). Interestingly, we did not observe estrogenic activity of *trans-*

**TABLE 1**

*Sperm count, sperm morphology, and serum hormones of control and trans-resveratrol-treated Sprague-Dawley male rats*<sup>1</sup>

Variable	Control group	Resveratrol group
<b>Spermatozoa</b>		
Sperm counts ( $\times 10^7$ )	14.1 $\pm$ 0.80	24.8 $\pm$ 3.30*
Total morphological abnormalities, %	12.5 $\pm$ 1.21	12.4 $\pm$ 1.13
Head abnormalities, %	0.62 $\pm$ 0.24	0.60 $\pm$ 0.16
Neck and midpiece abnormalities, %	6.38 $\pm$ 0.98	6.18 $\pm$ 0.70
Tail abnormalities, %	1.78 $\pm$ 0.23	1.54 $\pm$ 0.42
Distal cytoplasmic droplets, %	3.94 $\pm$ 0.69	4.68 $\pm$ 1.05
<b>Hormones</b>		
LH, $\mu\text{g/L}$	6.30 $\pm$ 0.77	10.65 $\pm$ 1.48*
FSH, $\mu\text{g/L}$	109.0 $\pm$ 14.2	244.3 $\pm$ 36.4*
Testosterone, nmol/L	12.51 $\pm$ 1.35	33.25 $\pm$ 9.85*

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 12$ . \* Different from the control group,  $P < 0.05$ .

resveratrol. Our results indicate that the daily oral administration of 20 mg/kg for 90 d did not affect body weight or food and water consumption in the treated group compared with the control rats. Given that growth inhibition is a sensitive indicator of estrogenic effects (16), this lack of reduction in body weight in the treated rats substantiates that *trans*-resveratrol does not act as an estrogen agonist, which is in agreement with other *in vivo* studies (17,18). Consequently, this compound could interact with the ER, thus increasing the secretion of gonadotrophins, leading in turn to an increment in testosterone and sperm output. Furthermore, the effects described above may have been enhanced through androgen antagonism because *trans*-resveratrol also antagonizes androgen action in prostate cancer cells by inhibiting AR activity and suppressing AR expression (19,20).

The behavior of *trans*-resveratrol as a mixed weak agonist/antagonist, without estrogenic properties, was also confirmed by the absence of a clear estrogenic effect on testes, as opposed to DES, a structural analog of *trans*-resveratrol and a potent estrogen agonist. In contrast to *trans*-resveratrol, DES has deleterious effects on the male reproductive tract. Rats treated with DES show adverse effects that include a reduction in testicular weight associated with impaired seminiferous tubular morphology (21,22). Exposure to DES also impairs spermatogenesis, which is substantiated at least in part by a reduced testosterone concentration (22,23). Although structurally similar, the distinct activity of *trans*-resveratrol and DES can be explained by subtle differences in their molecules. Compared with *trans*-resveratrol, DES lacks the 3-OH and 5-OH groups, but possesses a 4-OH group and 2 additional ethyl groups. These features provide differential binding characteristics to ER (24). DES has an affinity to ER similar to that of estradiol and acts as a potent agonist, which would explain the harmful effects described.

There is no information available in the literature on the activity of *trans*-resveratrol on male testes and spermatogenesis in either growing or adult rats. To our knowledge, the only 2 studies that we found were conducted in mice; one confirms the lack of harmful effects on testes (25), whereas the other describes a protective role in the male reproductive tract (26). Consequently, no data were provided concerning the effect of *trans*-resveratrol alone on spermatogenesis.

The effects of *trans*-resveratrol could also be mediated by counteraction of constitutive oxidative stress within the seminiferous tubules. It was shown previously that during spermatogenesis stages VI–VIII in rats, there is a significant increase in superoxide dismutase mRNA expression coinciding with the presence in the tubules of elongated spermatids with excess cytoplasmic retention. The cytoplasm was shown to produce high levels of reactive oxygen species (ROS) (27). *trans*-Resveratrol was found to be an effective scavenger of hydroxyl, superoxide, and metal-induced radicals as well as having antioxidant abilities in cells producing ROS. *trans*-Resveratrol exhibits a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS (1,2,14). Therefore, *trans*-resveratrol could be acting by decreasing the steady-state levels of ROS and proinflammatory factors in the seminiferous tubules, thus increasing sperm and androgen production. Together, these activities could also account for the increase in sperm output observed in healthy rats.

Our findings indicate that *trans*-resveratrol merits further research because this phytochemical may constitute a promising new compound for the treatment of male infertility. In Western society, infertility is a growing problem; its causes are diverse, and considerable effort is being made to provide effective therapy. In the case of male infertility, antioxidants, anti-inflammatory, androgens, and antiestrogens are some of the treatments used. However, a truly effective treatment has yet to be found (27).

In conclusion, we describe here a novel activity of the phytochemical *trans*-resveratrol. The daily oral administration of this compound to adult male rats enhanced spermatogenesis through the stimulation of the hypothalamic-pituitary-gonadal axis, and did not have adverse effects. These findings indicate that *trans*-resveratrol may provide a treatment for male infertility.

## LITERATURE CITED

- Bhat, K.P.L., Kosmeder, J. W. & Pezzuto, J. M. (2001) Biological effects of resveratrol. *Antioxid. Redox Signal* 3: 1041–1064.
- Aziz, M. H., Kumar, R. & Ahmad, N. (2003) Cancer chemoprevention by resveratrol: *in vitro* and *in vivo* studies and the underlying mechanisms. *Int. J. Oncol.* 23: 17–28.
- Hess, R. A., Bunick, D., Lee, K. H., Bahr, J., Taylor, J. A., Korach, K. S. & Lubahn, D. B. (1997) A role for estrogens in the male reproductive system. *Nature (Lond.)* 390: 509–511.
- Sharpe, R. M. (1997) Do males rely on female hormones? *Nature (Lond.)* 390: 447–448.
- Sharpe, R. M. (1998) The role of oestrogen in the male. *Trends Endocrinol. Metab.* 9: 371–377.
- Juan, M. E., Lamuela-Raventós, R. M., de la Torre-Boronat, M. C. & Planas, J. M. (1999) Determination of *trans*-resveratrol in plasma by HPLC. *Anal. Chem.* 71: 747–750.
- Juan, M. E., Vinardell, M. P. & Planas, J. M. (2002) The daily oral administration of high doses of *trans*-resveratrol to rats for 28 days is not harmful. *J. Nutr.* 132: 257–260.
- Anderson, J. E. & Thliveris, J. A. (1986) Testicular histology in streptozotocin-induced diabetes. *Anat. Rec.* 214: 382–387.
- Bamba, K. (1998) Evaluation of acrosomal integrity of boar spermatozoa by bright field microscopy using an eosin-nigrosin stain. *Theriogenology* 29: 1245–1251.
- Carreau, S., Bourguiba, S., Lambard, S., Galeraud-Denis, I., Genissel, C. & Levallet, J. (2002) Reproductive system: aromatase and estrogens. *Mol. Cell Endocrinol.* 193: 137–143.
- Crowell, J. A., Korytko, P. J., Morrissey, R. L., Booth, T. D. & Levine B. S. (2004) Resveratrol-associated renal toxicity. *Toxicol. Sci.* 82: 614–619.
- O'Donnell, L., Roberston, K. M., Jones, M. E. & Simpson E. R. (2001) Estrogen and spermatogenesis. *Endocr. Rev.* 22: 289–318.
- Couse, J. F. & Korach, K. S. (1999) Estrogen receptor null mice. What have we learned and where will they lead us? *Endocr. Rev.* 20: 358–417.
- Roemer, K. & Mahyar-Roemer, M. (2002) The basis for the chemopreventive action of resveratrol. *Drugs Today* 38: 571–580.
- Mueller, S. O., Simon, S., Chae, K., Metzler, M. & Korach, K. S. (2004) Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$  in human cells. *Toxicol. Sci.* 80: 14–25.
- Hart, J. E. (1990) Endocrine pathology of estrogens: species differences. *Pharmacol. Ther.* 47: 203–218.
- Turner, R. T., Evans, G. L., Zhang, M., Maran, A. & Sibonga J. D. (1999) Is resveratrol an estrogen agonist in growing rats? *Endocrinology* 140: 50–54.
- Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R. & Aou, S. (2003) Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci. Res.* 45: 345–356.
- Stewart, J. R., Artime, M. C. & O'Brien, C. A. (2003) Resveratrol: a candidate nutritional substance for prostate cancer prevention. *J. Nutr.* 133: 2440S–2443S.
- Gao, S., Liu, G. Z. & Wang, Z. (2004) Modulation of androgen receptor-dependent transcription by resveratrol and genistein in prostate cancer cells. *Prostate* 59: 214–225.
- Sharpe, R. M., Atanassova, N., McKinnell, C., Parte, P., Turner, K. J., Fisher, J. S., Kerr, J. B., Groome, N. P., Macpherson, S. et al. (1998) Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development. *Biol. Reprod.* 59: 1084–1089.
- Fritz, W. A., Cotroneo, M. S., Wang, J., Eltoum, I. E. & Lamartiniere, C. A. (2003) Dietary diethylstilbestrol but not genistein adversely affects rat testicular development. *J. Nutr.* 133: 2287–2293.
- Goyal, H. O., Braden, T. D., Mansour, M., Williams, C. S., Kamaleldin, A. & Srivastava, K. K. (2001) Diethylstilbestrol-treated adult rats with altered epididymal sperm numbers and sperm motility parameters, but without alterations in sperm production and sperm morphology. *Biol. Reprod.* 64: 927–934.
- Abou-Zeid, L. A. & El-Mowafy, A. M. (2004) Differential recognition of resveratrol isomers by the human estrogen receptor- $\alpha$ : molecular dynamics evidence for stereoselective ligand binding. *Chirality* 16: 190–195.
- Kyselova, V., Peknicova, J., Buckiova, D. & Boubelik, M. (2003) Effects of *p*-nonylphenol and resveratrol on body and organ weight and *in vivo* fertility of outbred CD-1 mice. *Reprod. Biol. Endocrinol.* 1: 30–40.
- Revel, A., Raanani, H., Younglai, E., Xu, J., Han, R., Savouret, J. F. & Casper, R. F. (2001) Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. *Reprod. Toxicol.* 15: 479–486.
- Haidl, G. (2002) Management strategies for male factor infertility. *Drugs* 62: 1741–1753.