# Review Article

## Lifestyle And Environmental Factors Influencing Male Fertility

### Siddharth C\*, Ramesh Raja D\*\*, Prathima T\*\*\*

\*Fellow in Clinical Andrology, \*\*Clinical Embryologist and Andrologist \*\*\*Embryologist, Dept. of Reproductive Medicine, Chettinad Hospital and Research Institute, Chennai, India.



Dr. C. Siddharth graduated in Medicine from J.J.M Medical College, Karnataka following which he pursued Masters in Clinical Embryology from Monash University, Melbourne. He worked as an Embryologist at Nova IVI Fertility Centre in Bangalore. He finished his Fellowship in Clinical Andrology from the Department of Reproductive Medicine, Chettinad Health City.

Corresponding author - C. Siddharth (c.siddharth@gmail.com)

## Abstract

The decline in the quality of semen parameters over the last few decades can be partly attributed to lifestyle and environmental factors. Recent trends in lifestyle including sedentary jobs, obesity, smoking, alcohol consumption, recreational drug abuse along with environmental factors like pollution and toxic chemical substances have been found to have adverse effects on the functional capacity of spermatozoa thereby impairing male fertility. A positive change in quality of life like healthy dietary habits, regular physical exercise and avoidance of smoking, alcohol intake and recreational drug abuse may reduce the damage incurred to the spermatozoa, thereby increasing the male fertility potential.

Key Words: Male infertility, Lifestyle, Environment, Altered spermatogenesis, Impaired function

Chettinad Health City Medical Journal 2014; 3(4): 156 - 160

## Introduction

The last few decades have seen a substantial decline in the quality of semen parameters<sup>1,2</sup>. This phenomenon may be partly attributed to the changes in the lifestyle factors and environmental changes. The lifestyle factors that influence male fertility include smoking, alcohol consumption, recreational drug use, stress, radiation, improper dietary habits and lack of physical activity leading to obesity. Environmental factors influencing male fertility include seasonal temperature variations and other occupational exposures. This review article aims to understand the mechanisms by which these lifestyle and environmental factors negatively influence male fertility.

## Effects of Temperature

Environment plays a very significant role in male fertility as it is well established that normal spermatozoa functions are affected by environmental changes<sup>3</sup>. In men, normal spermatogenic functions of the testis require the scrotal temperature to be steadily kept  $2 - 2.5^{\circ}$ C lower than the normal core body temperature. This thermoregulatory mechanism is maintained by the pampiniform plexus which acts as a counter current heat exchanger and by the scrotal dissipation of heat.

Persistent exposure to factors that compromise the thermoregulation capacity of the scrotum causes elevated scrotal temperature resulting in failure of spermatogenesis and reduced sperm numbers in the ejaculate<sup>4,5</sup>. Current day jobs, most of which are sedentary result in reduced air flow around the scrotum, leading to overheating in the same area and it has been suggested that this may have a significant adverse effect on the quality of spermatozoa that are produced<sup>6,7</sup>.

Long distance truck drivers, foundry workers, furnace workers, bakers, welders and those working in metal, glass and ceramic industries are particularly vulnerable to heat exposure. Continuous monitoring of temperature has revealed that scrotal temperature increases by  $1.7^{\circ}C$  - $2.2^{\circ}C$  within 2 hours of starting to drive a car<sup>5,7</sup>. These effects are also seen in paraplegic men who are confined to wheelchairs<sup>8</sup>.

However, the extent to which elevated scrotal temperature affects semen parameters is dependent on both the degree and duration of temperature elevation. Frequent scrotal exposures to relatively higher temperatures of >40°C (hot baths) for 20 minutes or more has higher chances of causing damage than smaller elevations in scrotal temperature ( $0.5^{\circ}C-1.0^{\circ}C$ )<sup>9</sup>. Frequent sauna baths, wearing of tight underwear for long duration of time and increased scrotal thickness which is seen in conditions such as elephantiasis of scrotum can also interfere with the thermoregulatory mechanism of the scrotum and thus cause spermatogenic abnormalities<sup>10</sup>.

Climatic variations could play an important role in sperm production as seasonal variations in sperm counts have been reported<sup>11</sup>. Reduced sperm concentrations have been consistently reported in the summer months which could be an indication of the adverse effect of the higher summer temperatures on sperm production<sup>12</sup>.

## Effects of Obesity

An increased likelihood of abnormal semen parameters like decreased spermatogenesis, poor sperm quality and reduced normal sperm morphology have been observed in men who are overweight thereby resulting in decreased fertility. Obesity thus should be considered as an important etiology of male infertility<sup>13</sup>. Obesity is also a direct result of emergent sedentary life style habits in young men<sup>14</sup>.

A Body Mass Index (BMI) of >25 kg/m2 has been shown to be negatively associated with sperm quality. For every 10 kilogram increase in a man's body weight above the corresponding normal weight, the chance of fertility is reduced by about 10%. Also, for every three point increase in the BMI, the risk of infertility increases by about 12%<sup>15</sup>.

The principal cellular constituents of adipose or fatty tissue are adipocytes whose proliferation is stimulated by estrogen while testosterone inhibits it. Also, adipocytes convert androgens to estrogens with the help of the enzyme aromatase. Hence, an increase in the adipose tissue mass causes an elevation in the circulating estradiol levels which in turn suppresses the Hypothalamus-Pituitary-Testicular axis, thereby reducing testosterone levels. The reduced testosterone levels further contributes to increased abdominal adipose tissue thus completing a vicious cycle.

Leptin, a hormone produced by fat cells is believed to play a role in the regulation of normal male reproduction. Male obesity is characterised by increased levels of leptin and hence leptin acts as a link between obesity and male infertility. Studies have shown the presence of leptin receptors in male germ cells, implicating its role in cell proliferation and differentiation<sup>16</sup>. It is believed that high leptin levels can disturb spermatocyte development and differentiation. A high leptin level also produces hypogonadism, reduces testosterone levels and induces testicular apoptosis<sup>17</sup>.

Obese men also carry the risk of testicular heat stress due to the accumulation of suprapubic and inner thigh adipose tissue<sup>18</sup>.

## Effects of Occupational Exposure

Men exposed to hazardous work environments which include handling of toxic or harmful chemical substances and radiations, run an increased risk than the general population of incurring damage to their sperms. Lead poisoning in battery plant workers can lead to fibrosis around the seminiferous tubules causing testicular damage<sup>19</sup>. Metals like cadmium, chromium and manganese can affect sperm motility and the effects are more so in steel welders who are exposed to these metals in larger amounts<sup>20</sup>.

Studies have reported decreased sperm motility and vitality in men working at toll booths and traffic policemen who are exposed to pollutants like nitrous oxide, sulphur oxide and carbon monoxide at significantly high levels<sup>19</sup>. Agricultural workers exposed to insecticides, pesticides (e.g., carbamates), Dibromochloropropane (agricultural soil fumigant) and workers in synthetic rubber industries(exposed to the plastic monomer chlorprene) have also been found to have low sperm counts<sup>21,22</sup>.

## **Effects of Alcohol**

The effects of alcohol were known way back in the

Elizabethan era when William Shakespeare rightly said, "Alcohol provokes the desire but takes away the performance" when describing the power of alcohol in relation to sexual function. Few of the ill effects of alcohol are decreased libido and disruption of normal spermatogenesis due to its effect on the pituitary or hormonal levels. Evidence suggests that alcohol can impair testosterone production and can cause shrinkage of testicular volume and also hinder erection<sup>23</sup>. To demonstrate the adverse effects of alcohol, a study was conducted among volunteers with normal liver function, who were administered 15% solution of alcohol every 3 hours over a 4 week period along with diet containing proteins, vitamins, folic acid and minerals and was found that there was a decline in testosterone levels<sup>24</sup>. Alcohol also disrupts normal spermatogenesis which has been shown in autopsy findings of alcoholics<sup>25,26</sup>. However, the exact mechanism by which alcohol affects sertoli cells and disrupts the normal functions is unknown<sup>27</sup>.

Excessive intake of alcohol also disturbs the hypo-thalamo-pituitary function, further worsening testicular and sexual function. Sexual dysfunction is a well-known consequence of alcoholism, as are the signs of hyperestrogenism which is probably secondary to disturbances of testosterone and estrogens metabolism in the cirrhotic liver<sup>28,29</sup>. Advancing age, decline in testosterone levels and increases in gonadotrophin levels are associated with a decrease in sperm production and number of normal sperm<sup>30</sup>.

## Effects of Smoking

"Cigarette smoking is injurious to health" is a statutory warning printed on all cigarette packets across the world in spite of which, number of smokers are steadily increasing worldwide. The effects of smoking, either active or passive or through third hand smoke (present in the environment), affects the respiratory, cardiovascular and reproductive system and produces various pathologies. It is of particular concern to the reproductive system as it affects the semen parameters.

Spermatozoa produced in men who smoke have reduced fertilising capacity and also produce defective embryos which have lower implantation rates. These occurrences are difficult to substantiate with evidence but a number of follow up studies have shown the lower implantation potential due to aneuploidy<sup>31-33</sup>.

Numerous authors have found a negative impact of smoking on human semen parameters negatively correlating with the number of cigarettes smoked per day and particularly the duration of smoking. Some authors have clearly stated that smokers demonstrate lower semen volume, sperm concentration, sperm motility and viability compared with non-smokers. They have also found that these semen shown an increased seminal leukocyte count and an increased incidence of globozoospermia<sup>34-38</sup>.

The causes for fertilization failure or poor implantation potential can be due to acrosomal damage or DNA damage during spermiogenesis<sup>39,40</sup>. Studies found that sperm DNA fragmentation in smokers is demonstrably high when compared with non-smokers<sup>41</sup>.

The hypo-thalamo-pituitary axis can be altered by nicotine through stimulation of growth hormone, cortisol, vasopressin and oxytocin release, which indirectly inhibit release of LH and prolactin.

Ochedalski et al reported that the mean level of 17 beta-estradiol was higher and the mean levels of LH, FSH and prolactin were found to be lower in smokers when compared with non-smokers, while the mean levels of testosterone and dehydroepiandrosterone did not differ<sup>42</sup>. Increased free and total serum testosterone and decreased prolactin levels were observed in smokers<sup>43</sup>.

The function of accessory sex glands in smokers has been assessed by determining the various glandular markers in the ejaculate such as N-acetyl amino sugar, total phosphate, zinc, acid phosphatase, and alpha-1,4-glucosidase. Both the vesicular and prostatic parameters were significantly lowered in smokers<sup>44</sup>.

The seminal ejaculates of smokers are varied in comparison to non-smokers, with the presence of Detached Ciliary Tufts (DCTs) and mast cells. Bornman et al, hypothesized that DCTs which originate from the epididymal epithelium may be shed as a result of testicular pathology and their presence has been demonstrated in the semen of smokers<sup>45</sup>. It has been shown that the seminal mast cells detected at higher frequency among smokers lead to reduced progressive motility of spermatozoa<sup>46</sup>.

## Effects of illicit drugs

The illicit drugs that can cause male infertility include marijuana, cocaine, methamphetamines, opioid narcotics and anabolic-androgenic steroids. These drugs are often abused by men during the reproductive years leading to compromised fertility.

Marijuana, when smoked releases a psychoactive cannabinoid compound called delta-9-tetrahydrocannabinol (THC). THC acts on the receptors present in the sperm middle piece and inhibit its mitochondrial activity resulting in reduced sperm motility<sup>47,48</sup>. Methamphetamines are associated with an increased incidence of sperm DNA damage<sup>49</sup>.

Anabolic-Androgenic Steroids are the most commonly abused drugs by sportsmen for performance enhancement. AAS users often take supraphysiological doses of testosterone which are 50 to 100 times greater than the normal production<sup>50</sup>. These exert a negative feedback on FSH, LH and endogenous testosterone secretion thereby impairing sperm concentrations<sup>51</sup>.

## Effects of stress

Though the impact of stress on infertility still remains unestablished, its aetiology cannot be completely overlooked. Several studies have stated that sperm motility and morphology are reduced due to emotional stress. One of the mechanisms could be due to negative impact on the pituitary gonadal axis thereby resulting in decreased sperm concentration and motility<sup>52</sup>. A study done in Turkey by Eskiocak, on 27 normal healthy men found that the activities of antioxidant enzymes present in semen like superoxide dismutase and catalase were increased when stress levels were high leading to increased production of free radicals. This increase can negatively impact the sperm parameters, mainly motility<sup>53</sup>.

Another important factor to be taken into consideration is the influence of stress on autonomous nervous system which in turn is responsible for ejaculation of semen. So, increased stress can cause decreased semen volume and also reduced sperm motility<sup>54</sup>.

## **Effects of radiation**

The cells in the human body, especially germ cells and Leydig cells are sensitive to different kinds of radiation including X-rays, gamma rays and mobile phone radiations. Numerous studies have established the impaired effects of radiofrequency electromagnetic waves (RF-EMWs) on sperm motility which act either through production of heat or through radiations, but which of the two has greater negative impact on semen quality still remains to be established. The thermal effects are most likely to affect sperm concentration as prolonged usage of mobile phones while placed in the trouser pocket, increases the scrotal temperature. The radiations (non-thermal) have been known to affect sperm motility and vitality<sup>55-57</sup>. Studies have also stated that there is an increase in DNA fragmentation due to increased reactive oxygen species production, one of the damages caused by radiation<sup>58</sup>.

## Conclusion

Apart from genetic, infectious, hormonal and idiopathic causes, male infertility due to lifestyle and environmental factors is an area of increasing concern. If the trends of increased smoking, alcohol intake and recreational drug use continue along with increasing trends in obesity, it is highly probable that male fertility could be severely compromised in the population in the years to come. However, with changes in lifestyle such as healthy dietary habits, regular physical exercise and avoidance of smoking, alcohol intake and recreational drug abuse, this can be prevented to a certain extent.

### References

- Giwercman A, Bonde JP. Declining male fertility and environmental factors. Endocrinology and metabolism clinics of North America 1998; 27(4): 807-830.
- 2) Carlsen E, Giwercman A, Keiding N, Skakkebæk NE. Evidence for decreasing quality of semen during past 50 years. BMJ 1992; 305(6854): 609.

Sinawat S. The environmental impact on male 3) fertility. Journal of the Medical Association of Thailand 2000; 83(8): 880.

Zorgniotti AW, MacLeod J. Studies in tempera-4) ture, human semen quality, and varicocele. Fertility and sterility 1973; 24(11): 854-863.

- 7) Thonneau P, Bujan L, Multigner L, Mieusset R. Occupational heat exposure and male fertility: a review. Hum Reprod 1998; 13: 2122-5
- Brindley GS. Deep scrotal temperature and the effect on it of clothing, air temperature, activity, posture and paraplegia. Br J Urol 1982; 54: 49-55
- 9) Setchell BP. Heat and the testis. Reprod Fertil 1998; 114: 179-84
- 10) Tiemessen CHJ, Evers JLH, Botys RSGM. Tight-fitting underwear and sperm quality. Lancet. 1996; 347: 1844-5
- Levine RJ. Seasonal variation of semen quality and fertility. Scandinavian journal of Work, Environment & Health 1999:34-37.
- 12) Gyllenborg J, Skakkebaek NE, Nielsen NC, Keiding N, Giwercman A. Secular and seasonal changes in semen quality among young Danish men - a statistical analysis of semen samples from 1927 donor candidates during 1977-1995. Int J Androl 1999; 22: 28-36.
- 13) Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010; 7(3): 153-161.
- 14) Kumar S, Kumari A, Murarka S, Kumar M. Lifestyle factors in deteriorating male reproductive health. Indian J Exp Biol 2009; 47(8): 615-24.
- 15) Sallmen M, Sandler DP, Hoppin JA, Blair A, Baird DD. Reduced fertility among overweight and obese men. Epidemiology. 2006; 17(5): 520-523.
- 16) Kay VJ, Barratt CL. Male obesity: impact on fertility. The British Journal of Diabetes & Vascular Disease 2009; 9(5): 237-241.
- Loret de Mola JR. Obesity and its relationship to infertility in men and women. Obstetrics and gynecology clinics of North America 2009; 36(2): 333-346.
- 18) Phillips KP, Tanphaichitr N. Mechanisms of obesity-induced male infertility. Expert Review of Endocrinology & Metabolism 2010; 5(2): 229-251.
- 19) Stachel B, Dougherty RC, Lahl U, Schlösser M, Zeschmar B. Toxic Environmental Chemicals in Human Semen: Analytical Method and Case Studies. Andrologia 1989; 21(3): 282-291.
- 20) Bonde JP. Semen quality in welders exposed to radiant heat. British Journal of Industrial Medicine 1992; 49(1): 5-10.
- 21) Lipshultz LI, Ross CE, Smith R, Joyner RE.
  Dibromochloropropane and its effect on testicular function in man. The Journal of Urology 1980; 124(4): 464.

- 22) Tielemans E, Burdorf A, te Velde ER, Weber RF, van Kooij RJ, Veulemans H, Heederik DJ. Occupationally related exposures and reduced semen quality: a case-control study. Fertility and Sterility. 1999; 71(4): 690-696.
- 23) Adler RA. Clinically important effects of alcohol on endocrine function. Journal of Clinical Endocrinology and Metabolism 1992; 74: 957–960.
- 24) Gordon CG, Altman K, Southren AL, Rubin E, Lieber CS. The effects of alcohol administration on sex hormone metabolism in normal men. New England Journal of Medicine 1976; 295: 793–797.
- 25) Pajarinen J, Karhunen PJ, Savolainen V, Lalu K, Penttila A, Laippala P. Moderate alcohol consumption and disorders of human spermatogenesis. Alcoholism: Clinical and Experimental Research. 1996; 20: 332–337.
- 26) Villalta J, Ballesca JL, Nicolas JM, Martinez de osaba MJ, Antunez E, Pimentel C. Testicular function in asymptomatic chronic alcoholics: Relation to ethanol intake. Alcoholism: Clinical and Experimental Research. 1997; 21: 128–133.
- 27) Zhu Q, Van Thiel DH, Gavaler JS. Effects of ethanol on rat Sertoli cell function: Studies in vitro and in vivo. Alcoholism: Clinical and Experimental Research. 1997; 21: 1409–1417.
- 28) Durphy BC, Burratt UR, Cooke ID. Male alcohol consumption and fecundity in couples attending and infertility clinic. Andrologia. 1991; 23: 219-221.
- 29) Tsujimura A, Matsumiya K, Takahashi T, Yamanaka M, Koga M, Miura H, Nishimura K, Takeyama M, Fujioka H, Okamoto Y, Iwamoto T, Okuyama A. Effect of lifestyle factors on infertility in men. Arch Androl. 2004; 50(1): 15-17.
- 30) Kalyani R, Basavaraj PB, Kumar ML. Factors influencing quality of semen: a two year prospective study. Indian J Pathol Microbiol. 2007; 50(4): 890-895.
- Soares SR, Simon C, Remohí J, Pellicer A.
  Cigarette smoking affects uterine receptiveness.
  Hum Reprod. 2007; 22(2): 543–7.
- Ramlau, Hansen CH, Thulstrup AM, Olsen J, Bonde JP. Parental subfecundity and risk of decreased semen quality in the male offspring: a follow-up study. Am J Epidemiol. 2008; 167(12): 1458–64
- 33) Rubes J, Lowe X, Moore D, Perreault S, Slott V, Evenson D, et al. Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril. 1998; 70(4): 715–23.
- 34) Hassa H, Yildirim A, Can C, Turgut M, Tanir HM, Senses T, et al. Effect of smoking on semen parameters of men attending an infertility clinic. Clin Exp Obstet Gynecol 2006; 33(1): 19–22.

- 35) Reina Bouvet B, Vicenta Paparella C, Nestor Feldman R. Effect of tobacco consumption on the spermatogenesis in males with idiopathic infertility. Arch Esp Urol 2007; 60(3): 273–7.
- 36) Gaur DS, Talekar M, Pathak VP. Effect of cigarette smoking on semen quality of infertile men. Singapore Med J 2007; 48(2):119–23.
- 37) Ramlau Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod 2007; 22(1):188–96.
- 38) Hassan A, Abo Azma SM, Fayed SM, Mostafa T. Seminal plasma cotinine and insulin-like growth factor-l in idiopathic oligoasthenoteratozoospermic smokers. BJU Int 2009; 103(1): 108–11.
- Lahdetie J. Micronucleated spermatids in the seminal fluid of smokers and non-smokers. Mutat Res 1986; 172(3): 255–63.
- 40) Sokol RZ, Mishell DR, Lobo RA, Sokol RZ.
  Pathophysiology of male infertility. The year book of infertility and reproductive endocrinology. Mosby Elsevier Health Science 1996: 88–102.
- Sepaniak S, Forges T, Fontaine B, Gerard H, Foliguet B, Guillet May F, et al. Negative impact of cigarette smoking on male fertility: from spermatozoa to the offspring. J Gynecol Obstet Biol Reprod 2004; 33(5): 384–90.
- 42) Ochedalski T, Lachowicz Ochedalska A, Dec W, Czechowski B. Examining the effects of tobacco smoking on levels of certain hormones in serum of young men. Ginekol Pol 1994; 65(2): 87–93.
- 43) Trummer H, Habermann H, Haas J, Pummer K. The impact of cigarette smoking on human semen parameters and hormones. Hum Reprod 2002; 17(6): 1554–9.
- Pakrashi A, Chatterjee S. Effect of tobacco consumption on the function of male accessory sex glands. Int J Androl 1995; 18(5): 232–6.
- 45) Bornman MS, Kok EL, du Plessis DJ, Otto BS. Clinical features of patients with detached ciliary tufts in semen. Andrologia.1989; 21 (1): 18–22
- 46) El Karaksy A, Mostafa T, Shaeer OK, Bahgat DR, Samir N. Seminal mast cells in infertile asthenozoospermic males. Andrologia. 2007; 39(6): 244–7.
- 47) Rossato M, Ion Popa F, Ferigo M, Clari G, Foresta C. Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. J Clin Endocrinol Metab. 2005; 90: 984–991.
- 48) Badawy ZS, Chohan KR, Whyte DA, Penefsky HS, Brown OM, Souid AK. Cannabinoids

inhibit the respiration of human sperm. Fertility and sterility 2009; 91(6): 2471-2476.

- 49) Barenys M, Macia N, Camps L, de Lapuente J, Gomez-Catalan J, Gonzalez-Linares J, Borras M, Rodamilans M, Llobet JM. Chronic exposure to MDMA (ecstasy) increases DNA damage in sperm and alters testes histopathology in male rats. Toxicol Lett. 2009;191: 40–46.
- 50) Parkinson AB, Evans NA. Anabolic androgenic steroids: a survey of 500 users. Medicine and Science in sports and Exercise.2006; 38(4): 644-651.
- 51) Bonetti A, Tirelli F, Catapano A, Dazzi D, Dei Cas A, Solito F, Magnati G. Side effects of anabolic androgenic steroids abuse. International Journal of Sports Medicine. 2008; 29(08): 679-687.
- 52) Said TM. Emotional Stress and fertility. Ind J Med Res. 2008; 128:228-230.
- 53) Sevgi Eskiocak, Ali Serdar Gozen, Ali Serkan Kilic, Sebahat Molla. Association between mental stress & some antioxidant enzymes of seminal plasma. Indian J Med Res. December 2005; 122: 491-496
- 54) Naomi Schneid-Kofman, Eyal Sheiner, Does stress effect male infertility? – A debate. Med Sci Monit. 2005; 11(8): SR11-13
- 55) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R.. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study.Fertility and Sterility. 2009; 92(4): 1318-1325.
- 56) Adams JA, Galloway TS, Mondal D, Esteves SC, Mathews F. Effect of mobile telephones on sperm quality: A systematic review and meta-analysis. Environment International. 2014; 70: 106-112.
- 57) Asha Benziger, Ramesh Raja D, Pandiyan N. Impact of Radiation emitted by mobile phone during call mode on the ejaculated semen. Paper presented at the Chettinad National Fertility Colloquium; Chennai; 2013 September.
- 58) De Iuliis GN, Newey RJ, King BV & Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PloS one 2009; 4(7): e6446.

160