Lifestyle and environmental contribution to male infertility

Richard M Sharpe

MRC Human Reproductive Sciences Unit, Centre for Reproductive Biology, Edinburgh, UK

This chapter is an overview of recent developments in our understanding and thinking about the importance and nature of environmental effects on sperm counts and fertility in the human male. This area is plagued by imperfect studies, not necessarily because of imperfect design but because of other 'uncontrollable' constraints. The available data, therefore, need to be placed in context and account taken of the limitations of our understanding or, more correctly, our ignorance. As we enter the new millennium, one of the saddest scientific aspects of human reproduction and infertility is our persisting ignorance about the causes and treatment of male infertility. With one notable exception (Y chromosome microdeletions) there has been little advance in our understanding of the causes of male infertility and its direct treatment over the past 20 years. Although most infertile men can now be offered the chance of fertility via ICSI, it is largely ignored that this does not represent treatment of the patient's infertility (which will persist unchanged), but is a means of circumventing the problem and leaving it for the next generation to tackle. There are many reasons for our ignorance about the causes of infertility, and some of these are outlined below in order to emphasise how this limits our ability to establish whether or not specific lifestyle and environmental factors do, or do not, affect human male reproductive function.

Factors that may influence the prevalence or susceptibility of the human male to adverse reproductive effects and our ability to detect them

Correspondence to: Dr Richard M Sharpe, MRC Human Reproductive Sciences Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9ET, UK

An important puzzle that impacts on male infertility is the poor efficiency of spermatogenesis in the human male when compared with most other mammals[1]. Human males also 'naturally' produce a high proportion (30–50%) of morphologically abnormal sperm that are probably incapable of fertilising under natural conditions. These two aspects of human spermatogenesis are put in perspective by considering that a normal adult man produces approximately the same number of morphologically

normal sperm per day as a hamster, despite there being more than a 10-fold difference in testicular size in these two species[1]. Though there is no suggestion that the poor efficiency of spermatogenesis in the human male is the result of lifestyle or environmental causes, commonsense suggests that it renders us more susceptible to such factors than animals with more efficient spermatogenesis. The generally low fertility of humans compared with animals is presumably also attributable, in part, to our poor semen quality.

The poor average quality of human semen, combined with the naturally great variation in semen quality between individuals and from ejaculate to ejaculate in the same individual[2], means that cross-sectional studies in men face an uphill task when attempting to establish whether or not occupation, lifestyle or other environmental exposures are able to affect sperm production or quality. To detect a small effect (*e.g.* a 20% decrease in sperm concentration) requires large numbers of men[3]. In practice, because of differences in age, race, reproductive status/history (*e.g.* whether or not vasectomized) and variation in exposure to the agent of interest, such studies require hundreds rather than tens of men. In turn, this makes such studies extremely laborious and expensive and acts as a deterrent to their application. It also rules out many possible studies, *e.g.* in workplaces with low numbers of employees. Ten years ago, I had several times heard it proudly proclaimed (by members of the chemical industry) that very few occupational or environmental exposures had any real impact on male reproductive function as only a handful of examples of such effects had been demonstrated, and only one of these [exposure to the nematocide, dibromochloropropane (DBCP)[4]] had caused a major decrease in sperm counts. For the reasons stated above, only catastrophic changes in sperm counts (such as that induced by DBCP) can actually be detected in the small size studies that are frequently undertaken, so the absence of evidence for a greater number of lesser effects could be misleading. What has changed this perception is the evidence to have emerged in the past decade suggesting that sperm counts in the general population may have fallen[5].

Another factor that should be kept in mind when considering environmental effects on sperm counts is our evolutionary origin. Unlike man, most mammals are seasonal breeders, an adaptation that ensures that fertility and conception are timed so that the young are born at a time of year favourable to their survival. Humans are considered to be non-seasonal mammals, but there is good evidence to suggest that residual effects of this 'seasonality' may exist. In the male, the consistently lower sperm counts reported in the summer months[6,7] could be a reflection of such seasonal changes. However, it is also possible that this phenomenon reflects an adverse effect of the higher summer temperatures on sperm production (see below). Whatever the explanation, any study of sperm counts has to take account of season, in addition to known confounders such as age and period of abstinence[2].

Despite the misgivings voiced above, in some centres over the past decade well-designed studies involving large numbers of men have begun to establish the relationship between occupation/environment, sperm counts and fertility in cross-sectional and longitudinal studies[3,8,9]. Because of their careful design and the use of appropriately large numbers of men, such studies are potentially able to identify relatively small differences in sperm counts between different cohorts of men. For example, one recent study compared sperm counts in two groups of ~175 young men in Denmark, one from a rural and one from an urban population[10]. The study showed a small (24%) but significantly higher median sperm count in men from the rural group, a difference that was initially dismissed as due to sampling differences. However, measurement of blood levels of follicle-stimulating hormone (FSH) and inhibin B (a marker of spermatogenesis that emanates from Sertoli cells) in the two groups also showed a significant difference between the two groups, the levels of inhibin B being higher and FSH levels lower in the group with the highest sperm counts. Thus, combination of blood hormone measurements and sperm counts can probably give more accurate insight into environmental influences on human testis function.

Lifestyle/environmental changes this century and male reproductive health

In searching for environmental/lifestyle influences on male reproductive function, a logical starting point is to look back over the last 50–100 years, as this time period has seen progressive changes in many aspects of our diet and lifestyle as well as to our environment, notably in its contamination with man-made chemicals (Table 1). At the same time, there has been a substantial change in the disease patterns of humans, particularly in Western countries. At the beginning of the 20th century, infectious diseases were the biggest cause of death and morbidity whereas today, as we live much longer, lifestyle and dietary factors have become more important. Our battle with infectious diseases means that we are now routinely exposed to substantially more therapeutic drugs (in particular, antibiotics) than was the case 50 or 100 years ago, so it must also be considered whether or not such exposures might affect sperm production or quality (Table 1). More recently, there has been a progressive change in how physically active we are, both occupationally and in our free time. Many more jobs are now sedentary and males of all ages spend more time on sedentary hobbies and less time on physical activity than their fathers did[11]. One general consequence of this trend is that being seated slightly impairs the ability of the scrotum to

Table 1 Occupational and lifestyle factors, with the potential to affect human male sperm production and fertility, that have altered substantially over the past 50–100 years

Potential mechanism for effect on sperm production/fertility	*Occupations that may be at risk*	*Lifestyle/societal factors that may have altered impact on men*
Heat (increase in scrotal temperature)	Bakers, welders, some workers in metal/glass/ceramics industries Sedentary jobs that involve many hours seated (e g drivers, computer operators)	Sedentary lifestyle, tight underwear or outer wear Switch from baths to showers will have been beneficial
Exposure to pesticides	Pesticide applicators (farmers, greenhouse workers, parks/highways operatives) Pest/timber control operators Pesticide producers Fruit/vegetable harvesters/packers	More intensive agriculture Introduction and expanded use of pesticides Modern pesticides are probably far safer than those in use > 20 years ago Increased consumption of animal fats may deliver a 'cocktail' of fat-soluble chemicals
Exposure to other chemicals	Workers involved in chemical manufacture and use Drivers, traffic wardens, roadway maintenance/cleaners	Greater time spent driving and in traffic queues Increased atmospheric pollution Increased consumption of animal fats may deliver a 'cocktail' of fat-soluble chemicals
Altered hormonal exposure	Workers involved in manufacture of contraceptive pill or other hormonal agents	Dietary changes may alter metabolism of endogenous hormones Consumption of meat from hormone-treated livestock (hormone residues in fat) Increased consumption of soy-containing products Increased exposure to 'environmental hormones' via food packaging/greater use of pre-packaged foods.
Recreational/performance-enhancing drugs	Widespread usage by many professional and amateur sportsmen	Increase in use of recreational drugs Body-image concerns have led to increased use of anabolic steroids, *etc*
Therapeutic treatments	Many more patients now survive cancer therapy that may adversely affect spermatogenesis	Antibiotics or other widely prescribed drugs may adversely affect spermatogenesis

thermoregulate, which over long periods of time may exert a significant adverse effect on the quality and quantity of sperm that are produced (see below and Table 1). There are several other changes in occupation and lifestyle that might be expected to impact adversely on sperm production (Table 1) though, in general, conclusive scientific evidence to confirm this expectation is lacking. Viewed overall, it seems unlikely that human male reproductive disorders could have remained untouched by these pervasive changes in Western countries, though the scale of such changes is unknown. Before considering the more important factors listed in Table 1, it is worthwhile considering two other recent developments that are having a substantial impact on the way that we now perceive disorders of male reproductive development and function.

Fetal/neonatal origins of reproductive dysfunction in the male in adulthood: testicular cancer and sperm counts

One advance in understanding of human diseases/disorders in general, has been the demonstration that susceptibility to a range of important disorders in adulthood (*e.g.* diabetes, obesity, cardiovascular diseases) may be altered by environmental/lifestyle factors acting during fetal life-or early infancy[12]. This thinking is being applied to male reproductive disorders, including low sperm counts and infertility[13]. A good illustrative example is the progressive increase in incidence of testicular germ cell cancer that has occurred throughout the Western world over the past century[14,15]. This is a disease of young men and is associated with reduced semen quality and reduced fertility[16]. Perhaps not surprisingly, therefore, the most important risk factors for testicular cancer are disorders of development of the reproductive system, especially those related to impaired androgen production or action (*e.g.* cryptorchidism, hypospadias[15,17]) and these congenital disorders can also be important risk factors for low sperm counts in adulthood (Table 2). Another risk factor is low weight at birth (intra-uterine growth retardation; IUGR), and this is of particular interest as it is IUGR that is also a key factor in increasing risk of diabetes, obesity and hypertensive disease in adulthood (Table 2)[12]. The fact that incidence of testicular cancer in many countries is doubling every 20–30 years (as in the UK) also points clearly to lifestyle/environmental, rather than genetic, causes. It is now being argued that testicular cancer represents the most extreme (and least common) manifestation of a syndrome that results from disordered reproductive development and which also manifests in less extreme cases (but with higher frequency) as subnormal sperm counts. If this hypothesis is correct, it suggests that the incidence of men with low sperm counts is set to increase in the years ahead in line with the increase in testicular cancer. In this regard, the evidence that sperm counts may be falling (see below) is, therefore, not entirely unexpected.

The factors responsible for the increase in incidence of testicular cancer and the possible decrease in sperm counts are unknown. Perhaps the most important clue is that the reported increase in both disorders appears to be related to year of birth[15,17]. In other words, each later year of birth is associated with a progressive increase in risk of developing testicular cancer or of having low/reduced sperm counts. For testicular cancer, this 'birth cohort' effect has been demonstrated for virtually all countries that report an increase in incidence of the disease (*e.g.* Europe[18]; North America[19]). This fits with the origins of testicular germ cell cancers, as >95% of cases are thought to arise from premalignant gonocytes (fetal germ cells) that have developed abnormally, persisted into adulthood and

Table 2 Factors acting in fetal and/or neonatal life that are identified as risk factors for the development of testicular cancer and low sperm counts in adulthood, with comments on the potential influence of lifestyle changes this century on these risk factors

Risk factor	*Potential environmental/lifestyle influences that may have changed in the past century*
Cryptorchidism	• Evidence that incidence has increased in past half-century, though data are not completely reliable • Disorder is related to impaired androgen production or action or abnormalities of testicular development, but causes are largely unclear
Intra-uterine growth retardation (IUGR)	• IUGR induced by maternal smoking • More IUGR babies survive to adulthood • Effect of administration of dexamethasone during late pregnancy?
Altered sex steroid exposure of fetus/neonate (reduced androgen and/or increased oestrogen)	• Maternal dietary changes (e g increased consumption of refined sugars, could alter sex steroid metabolism via effects on sex hormone binding globulin, SHBG) • Increased exposure to man-made environmental chemicals with weak oestrogenic and/or anti-androgenic activity

then been triggered to develop into a tumour[20]. The inter-relationships between cryptorchidism, hypospadias and testicular cancer, in terms of each being a risk factor for the other, and the fact that IUGR is a risk factor for all of these conditions (Table 2), emphasises the potential importance of events in fetal life in the development of male reproductive disorders. This applies also to sperm counts (Table 2) as it is recognised that fetal, and especially early neonatal, life are important times when Sertoli cells in the human testis proliferate[1,13]. As the number of Sertoli cells in the adult testis determines how many sperm are made per day[1], it can be appreciated that factors which acted perinatally to reduce Sertoli cell proliferation, could theoretically lead to reduced sperm counts in adulthood. The fact that Sertoli cells are key regulators of germ cell (gonocyte) and Leydig cell development[1,13], also provides a rational basis for why the various disorders listed in Table 2 are inter-related.

A 'birth cohort' related fall in sperm counts has been reported for the UK[21], France[22] and Denmark[23]. However, this is not a universal finding and studies in other countries are divided as to whether or not sperm counts have declined and, if they have, whether or not this is related to year of birth[5,17]. Differences in subject selection, semen analysis methods (especially lack of standardisation) and allowance for confounding factors probably explain much of this inconsistency, but geographical/regional differences in sperm counts cannot be ruled out. Such differences have been reported for the US[24,25], though because of lack of standardisation in the studies, these data should be viewed with caution. However, studies in France, where there is a nation-wide, standardised programme for subject recruitment for semen donation and analysis, have also reported

significant differences in the various regions[26]. Similarly, significant differences in average sperm counts in men from four European countries (Finland, Denmark, France and Scotland) have been established, the Finnish men having the highest and the Danish the lowest, sperm counts[7]. The latter study was confined to young (fertile) men whose partners were currently pregnant and used recruitment and semen analysis methods that were standardised rigorously in the four countries. The explanation for these geographical differences in sperm counts is not apparent, but emphasises that such factors have to be taken into account in studies that seek for occupational or lifestyle effects on sperm counts. In this regard, the possibility that some of the geographical differences in sperm counts might reflect genetic (ethnic) differences is potentially important when conducting studies in a country such as the US, where there is such a complex mixture of ethnic groups.

Heat exposure: occupational, lifestyle and seasonal influences

Elevation of scrotal temperature to normal core body temperature results in complete failure of spermatogenesis in man and most mammals[27,28]. Elevation of scrotal temperature has even been evaluated as a potential method of male contraception and shown to be clinically effective[29]. However, only recently have high quality studies begun to assess the importance of occupational and lifestyle exposures to heat on human sperm counts (reviewed by Thonneau *et al*[30]). From these studies, it is clear that constant exposure to any factor that compromises the ability of the scrotum to thermoregulate will result in an adverse effect on one or more aspects of semen quality. Occupational exposure to radiant heat (*e.g.* in bakers, welders, furnace workers, ceramics workers, *etc.*) can induce such effects, but so also can any occupation that requires prolonged sitting and thus reduction in air-flow around the scrotum[27,30], such as in taxi drivers[31]. The latter effect is probably seen at its worst in paraplegic men who are confined to wheelchairs[32]. In this regard, it is notable that several studies in the past that have investigated the occupations of infertile men have identified drivers as a significant 'at risk' group.

The magnitude of the effect of elevated scrotal temperature on semen parameters is both temperature- and time- (*i.e.* duration of temperature elevation) dependent. Exposure to a relatively high temperature (*e.g.* >40°C, as in moderately hot baths) for 30 min or more is likely to have major adverse consequences[28], but smaller elevations in scrotal temperature (+0.7–2.5°C) for prolonged periods are probably of greater clinical significance as they apply to much greater numbers of men[11]. Several recent studies that have investigated the effects of the types/tightness of underwear

and trousers on scrotal temperature and/or on semen quality in men leave little doubt that such factors can exert significant adverse effects[33,34]. Combination of tight clothing and a sedentary occupation is likely to compound such effects.

Depending on the degree of scrotal temperature elevation and its duration, semen quality may be affected in terms of increase in morphologically abnormal sperm, decreased sperm motility and a decrease in actual sperm numbers[11,28]. These effects can undoubtedly lead to impaired fertility, whether this is qualitative (*i.e.* increased 'time to pregnancy'[35]) or complete infertility in severe cases[28]. Of greater concern is evidence that indicates an adverse effect of scrotal temperature elevation on subsequent implantation, early embryo development and miscarriage rates in female animals after mating with an 'affected' male[28]. So far, little account has been taken of whether or not male factors, such as scrotal heating, might be a contributory factor to the high rate of early pregnancy loss in the human by altering DNA integrity of the sperm.

Mention has already been made of seasonal changes in human semen quality. The data provide convincing evidence that sperm counts and/or semen quality are consistently poorer in the summer months than in the winter[6,36,37]. Probably the most convincing data come from two sets of studies, one longitudinal and the other cross-sectional. In the former[38,39], sperm counts from the same men collected in summer and winter were compared and showed an average 30% reduction in sperm counts in summer versus winter. Similarly, a recent cross-sectional study of male partners of pregnant women in four European countries, that utilised standardised methods of subject recruitment and semen evaluation, showed that sperm counts were significantly and uniformly lower (~30%) in those men providing semen samples in the summer than in the winter[7]. This seasonal difference was equally evident in all four countries, despite the fact that there were consistent differences in average sperm counts between the four countries. Differences in abstinence period have been ruled out as explaining this effect as have several other factors such as smoking. However, heat might be a significant factor, as there are data for farm animals showing reduced semen quality in the summer months[28]. There is currently no consensus on this. It can be argued that because there is a similar fall in summer sperm counts in men from both temperate (Finland, Denmark, Scotland[6,7]) and more equatorial regions (New Orleans, USA[40]), then heat is unlikely to be the key factor. However, this argument does not take account of the possible counterbalancing effects of wearing looser clothing, use of air-conditioning[39], *etc.*, which may equalise heat exposure in men from different latitudes. On the other hand, the consistency of the average difference in sperm counts between summer and winter in the various studies is perhaps more persuasive of a universal underlying cause[39], such as a residual seasonal effect as mentioned above.

Exposure to pesticides

In surveys of the general public, exposure to pesticides is nominated as a major factor which they perceive as a significant risk to fertility. Scientific data to support this perception are lacking. Apart from the catastrophic effects of exposure to DBCP on human sperm counts already mentioned, evidence from other studies for a significant effect of exposure to other pesticides on sperm counts or male fertility is equivocal. Some studies identify agricultural workers as more at risk of infertility[41], but evidence that pesticides are to blame is usually not available. A series of studies in Denmark[42–45], some involving comparison of organic and non-organic farmers and others that have evaluated exposure to a wide number of pesticides, have demonstrated no evidence for a significant effect of pesticide exposure on hormone levels, sperm counts or fertility. Though this appears re-assuring, it is perhaps prudent to maintain a healthy suspicion of pesticides. One particular cause for concern is the high levels of a range of pesticides in human adipose tissue[46], including the presence of 'older' pesticides that are now restricted in use in Europe. The accumulation of lipid-soluble pesticides in fat also raises the spectre of trans-generational transmission of such compounds via mobilisation of fat stores during lactation and their transfer *en masse* from the mother to the breast feeding infant; theoretically, first-born infants of older mothers would be most at risk. There are considerable difficulties in evaluating such possibilities, but it emphasises again the importance of perinatal exposures as a potentially important period for the induction of effects on male reproductive potential. In this regard, perinatal exposures to hormonally active chemicals ('endocrine disruptors'), some of which are lipophilic, has received most attention.

Exposure to 'endocrine disruptors'

This has become a world-wide issue in the last decade, though there is little in the way of clinical data to affirm the theoretical concern that developmental, and possibly adult, exposure to such compounds could be responsible for the increase in incidence of testicular cancer and the possible fall in sperm counts[17]. Studies from animal experiments have also resulted in conflicting conclusions, so that at present there is no consensus view on whether such exposures are a serious concern as far as male fertility is concerned. Nevertheless, it is remarkable how many different routes of exposure to such compounds have 'developed' over the past 60 years or so (Table 1). The main focus of concern, other than exposure to pesticides that are hormonally active, has been various chemicals that are constituents of plastics (*e.g.* bisphenolic and alkylphenolic compounds, phthalates) and to which there is widespread

human exposure (especially for the phthalates) via many different routes. All of these compounds appear to be very weak oestrogens and, judged on this potency alone, perhaps do not pose a significant risk to sperm counts or fertility[17]. Recently, animal studies have shown that phthalates are quite potent anti-androgens[47], and although this is of greater concern it is likely that human exposure is below the levels that induce adverse effects. Nevertheless, the view emerging is that human exposure to such compounds should be minimised on grounds of prudence.

A related issue that has received far less attention is the hormone treatment of livestock to promote growth[48]. This practice was banned in Europe in 1981, but continues in the US, where it is routine to use extremely potent oestrogenic compounds (*e.g.* zearalone). These compounds can accumulate to some extent in fat, though to what degree such residues survive and enter the human body in active form is disputed[48]. Because of their potency, exposure to even tiny amounts of such oestrogenic compounds is a cause for concern. The fact that so much of 'modern diseases' such as obesity and heart disease in Western societies are associated with increased consumption of animal products, especially fats, is an intriguing coincidence as is the parallel increase in hormone-dependent diseases such as breast cancer (and arguably, testicular cancer) which remains unexplained[48]. The possibility that human exposure to growth-promoting hormones via animal fats could have contributed to such changes is speculation, but deserves to be investigated thoroughly. Such studies should also identify if such exposures are a cause for concern as far as sperm counts and male fertility are concerned.

Finally, another societal change that undoubtedly has the potential to affect male fertility is the increased use of hormones for sporting or body-image purposes (Table 1). The extent of such usage is disputed, but it is reckoned that a substantial proportion of young males will use such compounds at some stage. As this usage is generally clandestine, studies to identify accurately its impact on sperm counts and fertility are largely lacking or are difficult to evaluate[49]. From studies involving the administration of sex steroids for contraceptive purposes in men, it is likely that the short-term use of anabolic steroids will have an adverse effect on sperm counts and fertility, but whether such usage has any significant, long-term impact of an irreversible nature, is unknown[49]. If it does, then it can be predicted that such men will be presenting at infertility clinics with increasing frequency over the next decade.

Concluding remarks

I began this chapter by emphasising how our ignorance constrains our ability to estimate accurately the impact that environmental and lifestyle changes this century have had on sperm counts and male fertility. This

explains most of the uncertainty and equivocation in the various sections above and undermines attempts to draw definitive conclusions. Nevertheless, some conclusions and recommendations can be made based on what is clear, combined with simple common sense. For example, no-one will dispute our increased sedentation at work and at leisure and already there is reasonably good evidence that this is bad news for sperm counts. Therefore, much greater attention of studies in the male to changes in scrotal thermoregulation are warranted in order that we can establish with some certainty whether this has clinically significant effects on fertility or on damage to sperm DNA that might affect the viability and health of offspring. Similarly, increased consumption of animal produce in Western countries this century is beyond dispute and the fact that animal fats will carry with them an echo of (fat-soluble) compounds from the environment as well as compounds (such as growth promoters with sex steroid activity) administered to the animals, has to be a general cause for concern, perhaps especially in terms of trans-generational transfer of such compounds. It may well prove that such exposures are without significant effect on sperm counts and fertility in the human male, but our present position of ignorance prevents us from establishing if this is the case.

There is one worrying certainty to have emerged this century and that is the increase, year on year, in incidence of testicular cancer in Western countries. This trend tells us in no uncertain terms that something(s) in our environment and lifestyle is having a profound negative effect on early male reproductive development. Testicular cancer affects a minority of men, but the steady increase in its incidence can be viewed as a beacon, signalling to us that male reproductive health is getting steadily worse. We should take notice of this early warning system and set about dispelling the ignorance that currently prevents us from understanding how our modern lifestyle impacts on male fertility and its long-term preservation.

References

1 Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD (Eds) *The Physiology of Reproduction, 2nd edn* New York: Raven, 1994; 1363-434

2 World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn Cambridge: Cambridge University Press, 1999

3 Bonde JP, Giwercman A, Ernst E Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design problems. *Occup Environ Med* 1996, 53: 511–9

4 Whorton D, Foliart D. DBCP: eleven years later. *Reprod Toxicol* 1988; 2: 155–61

5 Irvine DS. Declining sperm quality· a review of facts and hypotheses *Baillière's Clin Obstet Gynaecol* 1997; 11: 655–71

6 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

7 Jorgensen N, Andersen A-G, Eustache F *et al.* Regional differences in semen quality in Europe. *Human Reprod* 2000; In press

8 Bonde JP, Joffe M, Danscher G *et al.* Objectives, designs and populations of the European Asclepios study on occupational hazards to male reproductive capability *Scand J Work Environ Health* 1999, 25 (Suppl 1): 49–61

9 Thonneau P, Abell A, Larsen SB *et al.* Effects of pesticide exposure on time to pregnancy: result of a multicenter study in France and Denmark. Asclepios study group. *Am J Epidemiol* 1999; 15: 157–63

10 Jensen TK, Andersson AM, Hjollund NH *et al.* Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 1997; 82: 4059–63

11 Hjollund NH, Bonde JP, Jensen TK, Olsen J Diurnal scrotal skin temperature and semen quality. *Int J Androl* 2000; 23: 309–18

12 O'Brien PMS, Wheeler T, Barker DJP (Eds). *Fetal Programming: Influences on Development and Disease in Later Life* London Royal College of Obstetricians & Gynaecologists Press, 1999; 1–483

13 Sharpe RM. Fetal and neonatal hormones and reproductive function of the male in adulthood. In: O'Brien PMS, Wheeler T, Barker DJP (Eds). *Fetal Programming: Influences on Development and Disease in Later Life*. London: Royal College of Obstetricians & Gynaecologists Press, 1999, 187–94

14 Adami H, Bergstrom R, Mohner M *et al.* Testicular cancer in nine Northern European countries. *Int J Cancer* 1994; 59: 33–8

15 Toppari J, Larsen JC, Christiansen P *et al.* Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996; 104 (Suppl 4): 741–803

16 Moller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. *BMJ* 1999; 318 559–62

17 Sharpe RM. Environmental oestrogens and male infertility *Pure Appl Chem* 1998; 70: 1685–701

18 Bergstrom R, Adami HO, Mohner M *et al.* Increase in testicular cancer incidence in six European countries a birth cohort phenomenon. *J Natl Cancer Inst* 1996; 88: 727–33

19 Zheng T, Holford TR, Ma Z, Ward BA, Flannery J, Boyle P. Continuing increase in incidence of germ-cell testis cancer in young adults: experience from Connecticut, USA, 1935–1992. *Int J Cancer* 1996, 65 723–9

20 Skakkebaek NE, Berthelsen JG, Giwercman A, Muller L. Carcinoma-*in-situ* of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int J Androl* 1987, 10: 19–28

21 Irvine DS, Cawood EHH, Richardson DW, MacDonald E, Aitken RJ Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 1996; 312: 466–71

22 Auger J, Kuntsmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 1995; 332: 281–5

23 Bonde JP, Jensen TK, Larsen SB *et al.* Year of birth and sperm count in 10 Danish occupational studies. *Scand J Work Environ Health* 1998; 24: 407–13

24 Fisch H, Goluboff ET, Olson JH, Feldshuh J, Broder SJ, Barad DH. Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril* 1996; 65: 1009–14

25 Paulsen CA, Berman NG, Wang C. Data from men in Greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. *Fertil Steril* 1996; 65: 1015–20

26 Auger J, Jouannet P. Evidence for regional differences of semen quality among fertile French men. *Hum Reprod* 1997; 12 74–5

27 Mieusset R, Bujan L. Testicular heating and its possible contributions to male infertility a review. *Int J Androl* 1995; 18: 169–84

28 Setchell BP. Heat and the testis *J Reprod Fertil* 1998; 114: 179–84

29 Mieusset R, Bujan L. The potential of mild testicular heating as a safe, effective and reversible contraceptive method for men *Int J Androl* 1995; 17: 186–91

30 Thonneau P, Bujan L, Multigner L, Mieusset R. Occupational heat exposure and male fertility: a review. *Hum Reprod* 1998; 13: 2122–5

31 Figa-Talamanca I, Cini C, Varricchio GC *et al.* Effects of prolonged autovehicle driving on male reproductive function: a study among taxi drivers. *Am J Ind Med* 1996; 30: 750–8

32 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

33 Parazzini F, Marchini M, Luchini L, Tozzi L, Mezzopane R, Fedele L. Tight underpants and trousers and the risk of dyspermia. *Int J Androl* 1995; 18: 137–40

34 Tiemessen CHJ, Evers JLH, Botys RSGM. Tight-fitting underwear and sperm quality. *Lancet* 1996; 347: 1844–5

35 Thonneau P, Ducot B, Bujan L *et al.* Effect of male occupational heat exposure on time to pregnancy. *Int J Androl* 1997; 20: 274–8

36 Politoff L, Birkhauser M, Almendral A, Zorn A New data confirming a circannual rhythm in spermatogenesis *Fertil Steril* 1989; 52: 486–9

37 Saint Pol P, Beuscart R, Leroy-Martin B, Hermand E, Jablonski W. Circannual rhythms of sperm parameters of fertile men. *Fertil Steril* 1989; 51: 1030–3

38 Levine RJ, Mathew RM, Chenault CB *et al.* Differences in the quality of semen in outdoor workers during summer and winter. *N Engl J Med* 1990; 323 12–6

39 Levine RJ, Brown MH, Bell M, Shue F, Greenberg GN, Bordson BI. Air-conditioned environments do not prevent deterioration of human semen quality during the summer. *Fertil Steril* 1992; 57: 1075–83

40 Levine RJ, Brown MH, Bordson BI, Stanley JM, Mathew RM, Starr TB. Deterioration of semen quality during summer in New Orleans *Fertil Steril* 1988; 49: 900–7

41 Strohmer H, Boldizsar A, Plockinger B, Feldner-Busztin M, Fiechtinger W. Agricultural work and male infertility. *Am J Ind Med* 1993; 24: 587–92

42 Larsen SB, Joffe M, Bonde JP. Time to pregnancy and exposure to pesticides in Danish farmers. Asclepios Study Group. *Occup Environ Med* 1998. 55 278–83

43 Larsen SB, Giwercman A, Spano M, Bonde JP. A longitudinal study of semen quality in pesticide spraying Danish farmers. The Asclepios Study Group. *Reprod Toxicol* 1998: 12: 581–9

44 Larsen SB, Spano M, Giwercman A, Bonde JP. Semen quality and sex hormones among organic and traditional Danish farmers. Asclepios Study Group *Occup Environ Med* 1999: 56. 139–44

45 Juhler RK, Larsen SB, Meyer N *et al.* Human semen quality in relation to dietary pesticide exposure and organic diet. *Arch Environ Contam Toxicol* 1999; 37 415–23

46 Olea N, Olea-Serrano F, Lardelli-Claret P, Rivas A, Barba-Navarro A. Inadvertent exposure to xenoestrogens in children. *Toxicol Ind Health* 1999; 15: 151–8

47 Mylchreest E, Sar M, Cattley RC, Foster PMD. Disruption of androgen-regulated male reproductive development by di(n-butyl)phthalate during late gestation in rats is different from flutamide. *Toxicol Appl Pharmacol* 1999; 156: 81–95

48 Andersson AM, Skakkebaek NE Exposure to exogenous estrogens in food. possible impact on human development and health. *Eur J Endocrinol* 1999; 140. 477–85

49 Huhtaniemi IP. Anabolic-androgenic steroids – a double edged sword. *Int J Androl* 1994; 17. 57–62