

## Secular variations in sperm quality: fact or science fiction?

Variações seculares na qualidade dos  
espermatozóides: fato ou ficção científica?

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**Abstract** *The debate concerning the possible degradation in human sperm quality began in the 1970s, was revived at the beginning of the 1990s and has continued to mobilize the scientific community ever since. After the meta-analysis by Carlsen et al. (1992) showing a decline in human semen quality over the last 50 years, several groups investigated the sperm characteristics of more or less homogeneous groups of men who had provided semen at the same center for 10 to 20 years. A significant decrease in sperm concentration was reported in some studies, but not in others. Meanwhile, there is an increasing number of reports suggesting that physical and chemical factors introduced and spread by human activity in the environment may have contributed to sperm decline. At the end of the 20th century the debate on declining semen quality is not closed. The lack of certainty and the serious consequences that such a decline would have on the fertility of human populations make this an important public health issue at the start of the 21st century. For this reason, intensive research should be developed in both fundamental and epidemiological domains, particularly in South America, where industrial and agricultural pollution pose a serious threat to the population.*

**Key words** *Male Infertility; Semen; Endocrine Disruptors*

**Resumo** *O debate sobre o possível declínio na qualidade dos espermatozóides humanos começou nos anos 70, foi reativado no início dos anos 90 e continua mobilizando a comunidade científica desde então. Desde a meta-análise realizada por Carlsen et al. (1992), demonstrando um declínio na qualidade do sêmen humano ao longo dos últimos 50 anos, vários grupos investigaram as características dos espermatozóides de grupos mais ou menos homogêneos de homens que forneceram amostras de sêmen para o mesmo centro ao longo de 10 a 20 anos. Alguns estudos, mas não outros, relataram uma diminuição significativa na concentração de espermatozóides. Enquanto isso, há um número crescente de relatos sugerindo que fatores físicos e químicos antropogênicos possam ter contribuído para a deterioração dos espermatozóides. Até o final do século XX, ainda não havia se encerrado o debate sobre a queda na qualidade do sêmen humano. A falta de certeza e as graves consequências que tal declínio teriam sobre a fertilidade de populações humanas tornam esta questão prioritária para a saúde pública no início do século XXI. Portanto, é necessário um enorme esforço de pesquisa básica e epidemiológica, particularmente na América do Sul, onde a poluição industrial e agrícola representa uma grave ameaça à população.*

**Palavras-chave** *Infertilidade Masculina; Sêmen; Desreguladores Endócrinos*

## The debate

### The origins of the debate

There has been continuous debate concerning the possible decrease in sperm quality in the last few decades since the publication of a study by Nelson & Bunge (1974). From then until 1992, several authors reported such changes but were unable to establish a clear cause (Bendvold, 1989; Bendvold et al., 1991; Bostofte et al., 1983; James, 1980; Leto & Frensilli, 1981; Menkveld et al., 1986; Osegbe et al., 1986; Osser et al., 1984; Smith et al., 1978). Conclusions were drawn from highly selected populations or populations of individuals seeking medical help for infertility. In these conditions, an observed decrease in sperm quality may have been due to changes caused by infertility treatment or might simply reflect selection bias and not the reality of a true biological phenomenon.

### The relaunching of the debate

It was a Danish publication that relaunched the debate in the public arena (Carlsen et al., 1992). This study was a meta-analysis dealing with data from 61 publications that had appeared between 1938 and 1990. It included data concerning the sperm characteristics of 14,947 men in good health, originating from most of the regions of the world. According to the authors, the mean volume of ejaculate was 3.4ml in 1940 and 2.7ml in 1990. Similarly, mean sperm cells concentration was found to have decreased from 113 million/ml in 1940 to 60 million/ml in 1990, a decrease of almost 50% over 50 years. This study rapidly stimulated many comments, criticisms and reanalysis (Bahadur et al., 1996; Becker & Berhane, 1997; Bromwich et al., 1994; Farrow, 1994; Fisch & Goluboff, 1996; Olsen et al., 1995; Saidi et al., 1999; Swan et al., 1997). Many biases were identified: the geographical diversity of the studies, recruitment of men for multiple reasons, the number of men recruited, which differed greatly between studies, missing data for certain periods, factors likely to affect sperm characteristics not taken into account and, dubious statistical methodology. However, the Danish study had the merit of raising several questions: for example, is this phenomenon real? If it is real, what are the causes and consequences?

Since the publication of the Danish meta-analysis, various laboratories have analyzed their own data retrospectively. One of the first such studies was performed in Paris (Auger et al., 1995). Since its creation in 1973, the CECOS

(*Centre d'Etude et Conservation des Oeufs et du Sperme*; Center for the Study and Conservation of Eggs and Sperm) has analyzed a homogeneous group of 1,351 fertile men, all potential sperm donors, with no change in the mode of recruitment or methodology used for analysis in 20 years. A significant decrease in the concentration of spermatozoa was observed in this population, from 89 million/ml in 1973 to 60 million/ml in 1992. Similarly, the percentage of motile sperm cells and the percentage of morphologically normal sperm cells, were found to have significantly decreased during this period. Multiple regression analysis demonstrated an annual decrease of 2.6% in sperm cells concentration, of 0.3% in the percentage of motile sperm cells and of 0.7% in the percentage of morphologically normal sperm cells. Many other studies have since been published, some suggesting that there has been a secular decline in sperm quality (Adamopoulos et al., 1996; Bilotta et al., 1999; Bonde et al., 1998; Comhaire et al., 1995; de Mouzon et al., 1996; Gyllenborg et al., 1999; Irvine, 1994; Irvine et al., 1996; Menchini-Fabris et al., 1996; Ulstein et al., 1999; Van Waeleghem et al., 1996; Younglai et al., 1998; Zheng et al., 1997; Zorn et al., 1999) and others finding no change in sperm quality over time (Acacio et al., 2000; Andolz et al., 1999; Benshushan et al., 1997; Berling & Wolner-Hanssen, 1997; Bujan et al., 1996; Emanuel et al., 1998; Fisch et al., 1996; Handelsman, 1997; Paulsen et al., 1996; Rasmussen et al., 1997; Suominen & Vierula, 1993; Vierula et al., 1996).

### Is the debate over?

It is currently difficult to draw definitive conclusions one way or the other in that the methodology of these studies is far from uniform and most are subject to various methodological biases. The first possible bias concerns the homogeneity of recruitment and the characteristics of the populations included in the studies. Ideally, the study population should consist of fertile men, potential candidates for sperm donation. In some cases, however, it consists of men consulting for infertility and changes over time in access to treatment or therapeutic attitudes render the conclusions dubious. The number of subjects included in each study is also important. The high level of variability within and between individuals in terms of sperm characteristics demands the inclusion of a large number of subjects per year, which is not the case in a certain number of studies. Other sources of bias relate to the methodology used for sperm analysis, which is

highly subjective, particularly in the absence of perfectly standardized procedures. The statistical methodology used is also an important determinant of the conclusions of this type of study. Indeed, the possible decline in sperm quality is not necessarily linear and continuous and therefore it may not be possible to model it using simple linear regression. Last, but not least, many confounding factors, such as the duration of sexual abstinence before sperm analysis, age and the season of sampling, have not been systematically taken into account in many of these studies. In addition, and independently of variations over time at a particular place, differences are observed in the mean value of sperm cells concentration according to geographic region on a worldwide scale (Fisch & Goluboff, 1996) and in some cases even within a single country (Auger & Jouannet, 1997). Thus, if the suggested decline in sperm quality is real, it is neither universal nor homogeneous.

All the studies claiming a decline in semen quality are retrospective and it always can be said that prospective studies in the general population would be much better. Prospective studies would certainly be invaluable in this domain and should be encouraged, but it would be very naive to believe that this would prevent all the biases of the retrospective studies. Indeed it is very likely that attempts to develop such prospective trials would encounter great reticence in the general population if the study required semen collection. Therefore, any prospective study in this domain would be subject to participation bias. Furthermore, prospective studies ought to last several decades and it makes little sense to wait for the completion of such studies before initiating research into the origin of the possible decline in semen quality.

### What are the causes?

There is currently no evidence that the possible decrease in quality of human sperm has a genetic origin. In contrast, many environmental, in the broadest sense of the term, physical, chemical and psychological factors have been identified as having a strongly deleterious effect on spermatogenesis. Some of these factors have increased during the course of the last fifty years, due to the agricultural and industrial development of mankind. Could such factors be responsible for a generalized decline in sperm quality over time?

### The testicle, direct target of many environmental factors

It was in 1977, following the publication of an article by Whorton et al. (1977) that we first became aware that the production of sperm cells by the testicle could be affected by chemical agents used by humans. The use of dibromochloropropane (DBCP), a nematocide employed on various tropical crops, including bananas in particular, was found to have rendered thousands of agricultural workers sterile in many countries worldwide (Slutsky et al., 1999). A paper had already been published, in 1961, showing that DBCP caused testicular atrophy in laboratory animals (Torkelson, 1961). It took more than 25 years and the declaration of these men that DBCP had rendered them sterile, before interest in human reproductive toxicology was awakened. Since the discovery of the effects of DBCP, many other chemical substances have been shown to have the potential to alter the principal characteristics of sperm in men, such as the number of sperm cells, their motility and their morphology (Bonde, 1996). These substances include pesticides such as chlordecone (Cohn et al., 1978), ethylene dibromide (Ratcliffe et al., 1987) and carbaryl (Wyrobek et al., 1981), solvents such as glycol ethers (Welch et al., 1988), carbon disulfide (Lancranjan, 1972), styrene (Jelnes, 1988) and 2-bromopropane (Kim et al., 1996), and heavy metals (Lancranjan et al., 1975). To this incomplete list, we should add the many other substances for which experimental evidence has demonstrated testicular toxicity in animals but for which data are not available for humans (Bonde, 1996; Sundaram & Witorsch, 1995). It should be borne in mind that of the 100,000 or so molecules produced and released into the environment by mankind, very few have yet been evaluated for reproductive toxicity.

In addition to chemical attack, we must also consider physical aggression, to which the testicle is particularly sensitive. Indeed, this organ is one of the most susceptible to the effects of ionizing radiation. The number of sperm cells is reduced by doses as low as 0.15 Gy (150mS) (Popescu & Lancranjan, 1975; Rowley et al., 1974). A second physical factor which the testicle is highly sensitive is to heat. Spermatogenesis requires the temperature in the scrotum to be at least 3°C lower than body temperature. An increase in scrotal temperature disturbs spermatogenesis (Mieusset et al., 1991). Prolonged exposure to sources of radiant heat may lead to significant changes in sperm characteristics (Figa-Talamanca et al., 1992; Thon-

neau et al., 1998). Other physical agents, such as high-frequency electromagnetic fields, may also affect testicular function, as was shown by an American study of military radar operators (Weyandt et al., 1996).

Finally, we should not ignore the effects of regular consumption of tobacco, alcohol or drugs, all of which are likely to affect spermatogenesis (Bonde, 1996; Multigner & Spira, 1997). Stress, which is very difficult to quantify, has also been put forward as a factor that may have a negative effect on sperm production (Fenster et al., 1997; Negro-Vilar, 1993). Major changes in sperm quality have been observed in populations following catastrophes such as the Kobe earthquake (Fukuda et al., 1996).

Clearly, a large number of environmental factors are likely to affect spermatogenesis in humans. However, most of the studies cited above were carried out in a professional environment, in circumstances in which the level of chemical and physical exposure is generally high. This accounts for a non-negligible proportion of the adult male population. In addition, given the widespread use of chemical substances, in particular, it is perfectly legitimate to raise questions concerning the consequences for the general population of their accidental or deliberate release into the environment.

#### **Is the testicle a target of endocrine disruptors?**

A recent hypothesis put forward to account for secular changes in sperm quality involves endocrine disruptors. This group of molecules includes a diverse and heterogeneous variety of natural and synthetic chemical substances likely to have adverse effects on individual organisms through primary effects on endocrine systems. These substances, via their hormonal estrogenic or anti-androgenic activities, are likely to interfere with gonad development in the fetus, and the postnatal function of the testicle (Sharpe, 1993; Sharpe & Skakkebaek, 1993).

It has been known for some time that some xenobiotics may act in a similar way to hormones (xenohormones), thereby affecting endocrine regulations. Estrogenic activity was first demonstrated for a range of man-made chemicals in 1938 (Dodds & Lawson, 1938). It has been known since the 1960s that synthetic compounds such as the chlorinated insecticides methoxychlor and DDT, and polychlorinated biphenyls (PCBs) may have estrogenic activity in laboratory animals (Bitman et al., 1968; Bitman & Cecil, 1970; Tullner, 1961). The list of chemical substances with hormonal ac-

tivity *in vitro* or *in vivo* has not stopped growing in the last few years. In addition to those already mentioned, they include insecticides (chlordecone, lindane), fungicides (vinchlozoline), surfactants (alkylphenols), plastics (bisphenol-A, phthalates) and industrial by-products (dioxins) (reviewed in Colborn et al., 1993; Toppari et al., 1996).

However, it was the use of diethylstilbestrol (DES), a synthetic estrogen, with catastrophic consequences in humans, that led to the concept of endocrine disruption. DES was prescribed, between the end of the 1940s and the start of the 1970s, to thousands of pregnant women with a history of spontaneous abortion. Many studies were carried out on the consequences for health in adult life of the exposure of the children involved to DES in the uterus. These studies showed a decrease in the number and motility of sperm cells and a high percentage of morphologically abnormal spermatozoa in the sons of women treated with DES (Gill et al., 1977, 1979). Experiments *in vivo* in laboratory animals have shown that the administration of chlordecone, methoxychlor, octylphenol, butyl phthalate or dioxin during gestation or lactation causes a significant decrease in sperm production in the adult (Gray, 1982; Gray et al., 1989; Mably et al., 1992; Sharpe et al., 1995).

Several observations support (but without providing a clear demonstration) the idea that endocrine disruptors may be involved in changes for sperm quality in humans. In recent years, we have seen an apparent increase in the incidences of various specific diseases of the male reproductive apparatus, such as testicular cancer (Adami et al., 1994; Boyle et al., 1987; Pike et al., 1987; Spitz et al., 1986; Stone et al., 1991; Wilkinson et al., 1992), and developmental abnormalities such as cryptorchidism (Campbell et al., 1987; Chilvers et al., 1984) and hypospadias (Czeizel et al., 1986; Kallen et al., 1986; Matlai & Beral, 1985). The sons of women treated with DES were also found to have an abnormally high incidence of hypospadias, microphallus and cryptorchidism (Henderson et al., 1976). To these results in humans, we should add a collection of observations showing major changes in male reproductive function in wild animals (reviewed in Colborn et al., 1993; and Toppari et al., 1996). For example, the contamination with organochlorine insecticides of Lake Apopka in Florida in 1980 led to an abnormally high level of developmental abnormalities in the genital apparatus of the male alligator population (Guillette et al., 1994). Florida panthers have low ejaculate volumes, low sperm

counts, and a high proportion of abnormal sperm cells (Facemire et al., 1995). These effects were associated with the presence of environmental pollutants with estrogenic activity in the diet of these animals.

It is only recently that studies concerning secular changes in sperm quality in domestic animals have been carried out. A meta-analysis of published data from 1932 to 1995 suggested no significant change sperm concentration in bulls, boars and rams (Setchell, 1997). A Dutch long term study in dairy bulls, issued from a single artificial insemination center from 1977 to 1996, indicated no decline in sperm concentration during the study period (Van Os et al., 1997). A French study reported data on secular trends in semen quality in stallions over the period 1981 to 1996 (Multigner et al., 1999). A slight but significant decline in semen volume was observed, but total sperm production did not change. These studies do not confirm observations in humans. However, the procedures for selecting domestic animals should be taken into account. In most cases, the best reproducers are selected and this procedure may counteract a possible decline in sperm quality over time.

Although biologically plausible and supported by experimental data and observations in wildlife, there is no solid proof that environmental exposure to endocrine disruptors is the cause of reproductive disorders in humans. The multiple means of exposure to these substances (professional, food, air, water) and the diversity of their potential effects on health render their epidemiological evaluation particularly difficult.

### The state of affairs in South America

All of the studies carried out on secular changes in sperm quality have taken place in the northern hemisphere or Australia. To our knowledge, no longitudinal studies concerning secular changes in sperm quality have been carried out in South America. However, a study carried out in Venezuela, on the men in couples consulting for infertility suggested that there was no change in the proportion of men presenting azoospermia or oligospermia during the period between 1981 and 1995 (Tortolero et al., 1999). Unfortunately, this study was subject to a major recruitment bias, rendering extrapolation to the general population impossible. Similarly, no cross-sectional study linking environmental factors with sperm quality in humans in South America has been published.

A study has recently been carried out evaluating the impact of chemical exposures on the

sperm characteristics of populations of men consulting for infertility during the period 1995-1998 in the southern coastal region of Argentina (Oliva et al., 2001). This region encompasses the provinces of Entre Rios and Santa Fe, which have intense agricultural and industrial activity. It is therefore not surprising that almost 40% of the men consulting for infertility had been exposed to pesticides or solvents. Men exposed to pesticides or solvents were compared with those who had not been exposed to either. The concentration of sperm cells and the percentage of motile sperm cells were significantly lower in the men exposed to pesticides. In men exposed to solvents, the percentage of motile sperm cells and the percentage of morphologically normal sperm cells were significantly lower. These results remained significant after adjustment for age, duration of abstinence and the taking into account of various confounding factors. This study confirms the major role of environmental factors (in this case, in the professional environment of adults) as a risk factor for changes in sperm quality.

All evidence suggests that South America has the same environmental pollution as other regions of the world. Diverse studies have been carried out, principally in Brazil and Argentina, demonstrating the presence of chemical pollutants in various natural environments. The most studied, and most looked for, of these pollutants were pesticides. The presence of various organochlorine pesticides, such as DDT and lindane, has been demonstrated in water, sediment, freshwater fish and molluscs and in fruits from various regions of Brazil, both urban and rural (Araujo et al., 1999; Avelar et al., 1991; Caldas et al., 1999; Oliveira et al., 1995). Organochlorines have also been found at the end of the food chain, in human milk (Beretta & Dick, 1994; Matuo et al., 1992). In Argentina, PCBs and organochlorine insecticides have been detected in fish from Mar Chiquita (Menone et al., 2000) and in dairy products (Leonard et al., 1994, Maitre et al., 1994). It is not only urban areas and zones of intense agricultural activity that have been found to be contaminated, as shown by the presence of organochlorines in more isolated zones such as the Antarctic zones of Argentina, close to the Almirante Brown base (Garcia-Fernandez et al., 1979). All these examples illustrate the reality of contamination of diverse environments in South America and the ease with which molecules suspected to have endocrine disruptor activity diffuse and come into contact with human populations.

## Conclusions

One of the predictable consequences of a possible decrease in sperm quality in humans is an increase in the number of infertile couples. It is true that in many countries, an increase has been reported in the number of couples seeking medical help for difficulties in conceiving a child. Nevertheless, this increase is largely associated with the increase in accessibility of such treatment and with the development of treatments to facilitate procreation, and consequently, an increase in the number of medical consultations. It is therefore difficult today to evaluate the consequences for the population as a whole of a decline in sperm quality. A study recently carried out in Sweden observed no increase in subfertility (defined as a failure to conceive in one year of trying) during the period 1983-1993 (Akre et al., 1999). Similarly, a British study observed no change in the time required to achieve conception in a British population of 1,540 couples who conceived their children between 1961 and 1993 (Joffe, 2000). These studies are reassuring, but the small number of such studies makes it impossible to draw firm conclusions. In addition, the consequences of a decline in sperm quality may be temporarily counteracted by improvements in the health status of these same populations, due to a decrease in sexually transmitted diseases, for example. All this shows that it is still far from clear whether there has really been a decrease in sperm quality in humans and what the effects of such a decline are likely to be. However, this is no reason to ignore the reality of a certain number of facts. It is well estab-

lished that spermatogenesis is particularly sensitive to environmental attacks, physical or chemical in nature. The existence of substances with hormonal activity and their ability to modify subtle balances at various stages critical to the development of the reproductive apparatus is a potential danger that we should not underestimate.

It is therefore becoming urgent to develop new research on both fundamental and epidemiological aspects. Surveys in human populations are absolutely necessary to determine precisely the role of various environmental factors in male reproductive function. Exposed/non-exposed studies, one of the various methodological approaches possible (Schrader & Kanitz, 1994), are the most suitable for evaluating causal relationships between exposure and consequences for health. This type of study, however, requires the precise measurement, both qualitative and quantitative, of exposure.

Most of the studies devoted to the decline in sperm quality and the deleterious consequences of environmental factors on male reproductive function were carried out in highly developed countries (Europe, United States, Australia). South America is notable for its absence in this area of research. The industrial development and intensive agricultural activity of the South American continent, together with frequent non-respect of environmental protection measures, are a major threat to the health of human populations. Raising awareness of these problems and the implementation of research should be priorities for all the countries of South America.

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Submitted on 7 November 2000

Final version resubmitted on 4 April 2001

Approved on 2 May 2001