

**‘Mum... Is Great Grandma responsible for my low sperm count?’**

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**Introduction**

The last half century has seen a dramatic increase in the incidence of some diseases associated with the male reproductive system. In the majority of industrialised countries in North America, Europe and Oceania the incidence of testicular cancer (TC) has more than doubled over the last 30 to 40 years. Despite the potential bias of improved detection and data collection and issues of standardisation of diagnosis these are not thought to be sufficient to explain the apparent increase (Huyghe *et al.* 2003). Although a more controversial area it is also thought that over the last 50 years there has been a decline in sperm quality, at least in some geographical locations. Hypospadias and cryptorchidism, developmental defects of the urethra and scrotum respectively, are also thought to be on the increase.

As a result of these epidemiological findings, hypotheses have been suggested as to the aetiological factors causing these male reproductive defects. One recurring theme is the concern that certain environmental chemicals may play a role.

The global production of chemicals has increased from 1 million tonnes in 1930 to 400 million tonnes today, and there are approximately 100,000 different substances registered for commercial use in the EU (Strategy for a future Chemicals Policy, White Paper by the European Commission 2001). As a result there are an increasing number of synthetic compounds in our environment to which humans are being exposed to daily. These chemicals can be found in food packaging, agricultural chemicals and household detergents.

Is there a link between these increasing exposure to environmental chemicals and the increasing incidence of certain diseases or is it merely a coincidence? This essay will now go on to explore the effects of environmental chemicals on male reproductive systems and consider how this exposure may result in disease in humans.

## **Endocrine disruptors**

There is compelling evidence that some of these environmental chemicals contain compounds which can have hormone-like effects on the body. These chemicals, called endocrine disruptors (EDs), have the capacity to interfere with normal signalling systems and may mimic, block or modulate the synthesis, release, transport, metabolism and binding or elimination of natural hormones (Caserta *et al.* 2008). Although adult exposure to EDs is important, embryos and fetuses are the primary concern because EDs can cross the placenta and affect developing organisms at much lower levels than would be considered harmful in the adult. They are particularly sensitive to perturbation by chemicals with hormone-like activity and are therefore very vulnerable targets. More specifically reproductive health may be particularly sensitive to EDs because hormones play crucial regulatory roles in the initial development of reproductive tissues (Mantovani 2002).

Numerous animal and human studies have demonstrated reproductive alterations as a consequence of intrauterine and/or neonatal exposure to EDs. Examples include maternal exposure to phthalates (used in the plastic industry) causing adverse reproductive development of male offspring (Swan *et al.* 2005) and prostatic changes in fetuses of pregnant mice exposed to oestrogenic chemicals (Timms *et al.* 2005).

Perhaps more disturbingly however recent research has shown that EDs may do more than pose risks just to the offspring of mothers exposed during pregnancy: the effects may be transgenerational

## **Crossing generations**

Anway *et al.* (2005) exposed pregnant rats to two common environmental toxins; methoxychlor (an oestrogenic pesticide) and vinclozolin (an anti-androgenic fungicide). During days 8 to 15 of pregnancy (the time of gonadal sex determination) the gestating female (F0) rats were given intraperitoneal injections of one of the two chemicals. Thus the F0 gestating female, the developing embryo (F1 generation) and its germ cells (sperm of the F2 generation) were transiently exposed to the ED.

As expected from results of previous studies there were certain reproductive defects in the offspring. Although the offsprings' testes appeared normal and they could reproduce they had 20% lower sperm counts, sperm motility was 25-35% lower, sperm capacity was reduced and the cells within the testes underwent higher rates of apoptosis when compared to controls. The research was continued by breeding

the F1 males with females from different litters and subsequent breeding continued for four generations. Adult males from F1, F2, F3 and F4 generations were killed between postnatal days 60-180 for sperm and testes examination. Bearing in mind that only the original gestating mother (F0) was directly exposed and F1 (embryo) and F2 (germ cells) were indirectly exposed to the ED, it was very surprising that the defective F1 phenotype was passed through all generations up to F4. Thus irrespective of the type of ED exposure (direct in F1 to zero in F4) males from all generations (F1-F4) had reproductive organ changes i.e. the changes were transgenerational.

This transgenerational phenomenon was later defined as the ‘ability of an acquired physiologic phenotype or disease to be transmitted to subsequent generations through the germ line, despite the subsequent generation not being directly exposed to the toxicant’ (Skinner 2007). This startling finding showed support for the controversial idea that EDs could cause population wide reproductive problems.

### **Transgenerational transmission**

The ability of an external agent to induce a transgenerational effect requires a stable DNA/chromosomal alteration or an epigenetic phenomenon (Rakyan & Whitelaw 2003). If the transgenerational signal was due to direct DNA damage, Mendelian rules of genetics would apply and further mating of the exposed F1 offspring to control animals would result in an overall reduction in mutation rate in subsequent generations (Barber *et al.* 2002). However Anway *et al.* (2005) found that over 90% of males for all generations had the defective phenotype, with each generation having a similar rate, which significantly exceeded that of the control parents. This implicates an epigenetic mechanism as being responsible for the transgenerational effect seen through permanent reprogramming of the germ line.

### **Epigenetics**

Our epigenetic code lies on top of our genetic code as if it is ‘written in pencil in the margins around the DNA’ (Gosden & Feinbery 2007) and changes to it lead to alterations in DNA function without alterations to the DNA sequence (Jones & Takai 2001). Each cell type in our body has its own epigenetic signature which reflects genotype, developmental history and environmental influences and is ultimately reflected in the phenotype of cell and organism (Morgan *et al.* 2005). Mammalian epigenetics are generally stable, but there are periods during our early development when our epigenetics are reprogrammed; germ cell development is one of these periods. This epigenetic reprogramming is necessary to regulate crucial aspects

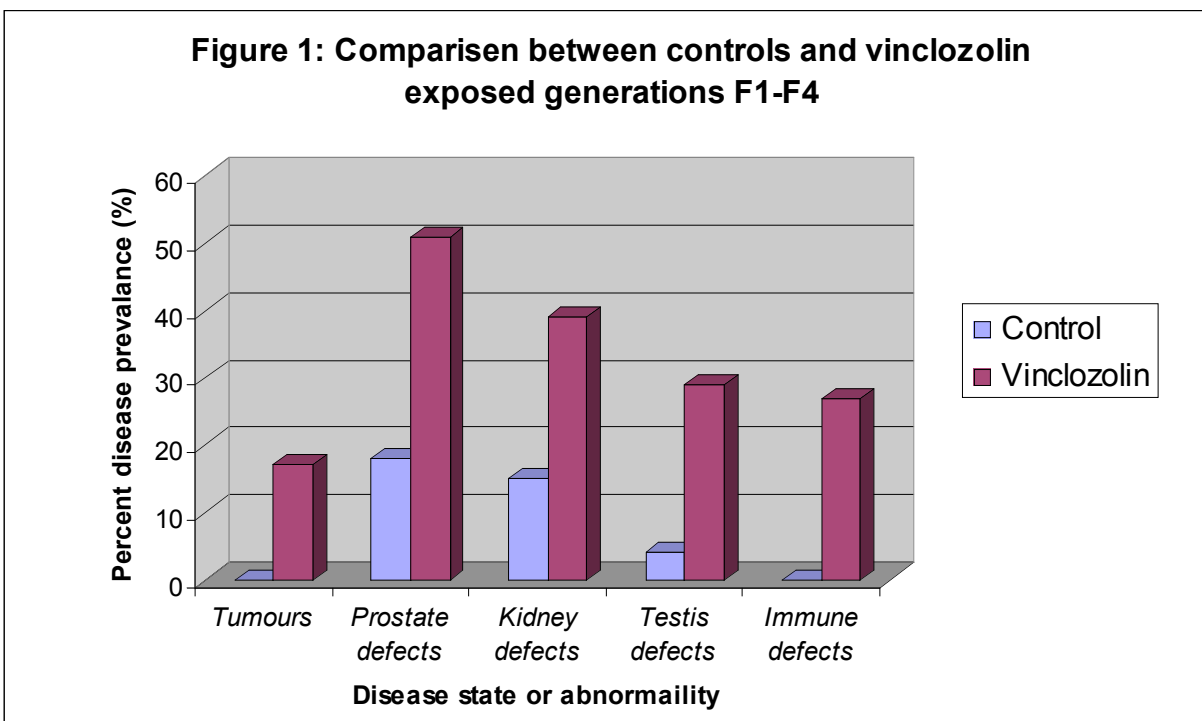
of the genome's function such as generating totipotency (the ability of a newly fertilised egg to differentiate into all tissues) and to erase acquired epigenetic information (Reik *et al.* 2001).

During germ cell development the fetal testis contain steroid receptors through which oestrogenic and androgenic substances can act. If during this critical germ line epigenetic reprogramming there is ED exposure, the chemicals could act via these steroid receptors and alter the DNA methylation process (removal and addition of methyl groups to DNA resulting in the turning on or turning off of a gene). Thus the germ line (sperm) can at least experimentally be modified by environmental toxicants through this epigenetic mechanism.

Once established the epigenetic state is maintained throughout the life of the individual and, upon breeding, results in transgenerational transmission of an altered phenotype. Anway provides the first experimental evidence that the effects of EDs can be transmitted transgenerationally via an epigenetic code.

### **Adult-onset disease**

The study by Anway *et al* (2005) led to the hypothesis that embryonic exposure to an ED at the time of gonadal sex determination may lead to transgenerational disease states in adults. Thus Anway and his team recreated the original experiment but allowed the F0-F4 generations to reach 6-12 months of age (Anway *et al.* 2006). The original observations were confirmed but also showed a more extensive disease phenotype in the older adult animals. When compared to controls there was an increased incidence of disease states and/or tissue abnormalities which developed at consistent rates in all exposed generations (F1-F4). These included prostate disease, kidney disease, immune abnormalities, testis abnormalities, and tumor development (see figure 1: the absence of a bar in the control group indicates zero).



The toxicology of vinclozolin in the environment is yet to be established and although the concentrations used here are likely to be higher than those in the environment the findings offer a novel mechanism for disease aetiology suggesting that the hazards of environmental toxins may be more pronounced than expected.

### **Conclusion**

The ground-breaking research by Anway *et al* (2005 and 2006) has provided a possible causal link between the increasing incidence of certain male reproductive diseases and increasing human exposure to environmental chemicals. The ability of an environmental factor to promote a variety of different disease states or abnormalities at high frequency for multiple generations is a new finding not previously appreciated. It is a feasible and appealing explanation: however evidence for the association between levels of exposure found in the general population and serious adverse effects on male reproduction is still lacking (Giwercman *et al* 2007).

The possibility that the effects are transgenerational has huge implications for future generations. If transgenerational epigenetic changes are responsible, they are irreversible, and we will continue to see ever increasing rates of disease. While further research into the role of epigenetics is required, it is hard not to see the implications as alarming.

At a population level, the only way of knowing if these mechanisms are responsible is if we significantly reduce our exposure to EDs and still observe an increase in disease incidence. Worldwide, governments are trying to reduce chemical usage and fully explore their potential adverse effects, but it might be too late. Unfortunately, only time will tell us whether Great Grandma really is responsible for causing low sperm counts in her great grandsons.

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