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Associate editor's comment on the article 'Semen quality of fertile US males in relation to their mothers' beef consumption during pregnancy' by Swan *et al.*

Could hormone residues be involved?

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In this issue, Swan *et al.* report finding a relationship between the amount of beef consumed by women during pregnancy and subsequent sperm concentration in their sons in adulthood. There is extensive evidence that maternal nutrition and maternal consumption of specific nutrients, drugs and chemicals present in food during pregnancy and lactation can have consequences for subsequent pathophysiology of offspring. This has been demonstrated experimentally in animals, and the developmental origins of human health and disease (DoHAD) hypothesis also has considerable support from the epidemiological literature (Fowden *et al.*, 2006; Gluckman *et al.*, 2007).

Only recently have researchers interested in fetal nutrition and metabolic diseases become aware of findings relating developmental exposure to the class of environmental chemicals known as endocrine disruptors to metabolic and reproductive disorders in males. Specifically, Skakkebaek *et al.* (2001) have identified that a cluster of reproductive abnormalities in males (sperm quality and testicular cancer) is associated with conditions identifiable at or shortly after birth (cryptorchidism, hypospadias), which they have named the 'testicular dysgenesis syndrome (TDS)'. This cluster of abnormalities of male reproductive development is proposed to be due, at least in part, to fetal/neonatal exposure to environmental estrogens (EE) [one example of such an EE is bisphenol A, the chemical used to make polycarbonate plastic and the resin lining of metal cans (vom Saal and Hughes, 2005)] and chemicals with anti-androgenic activity [one example would be phthalates used in PVC plastic and many other products (Swan *et al.*, 2005)]. Skakkebaek's hypothesis is thus that developmental disorders identifiable at birth are predictive of subsequent male reproductive system pathologies, such as reduced fertility due to low sperm count as well as testicular cancer.

Although there are a number of potential factors that could account for the association reported in the current study by Swan *et al.*, these authors suggest based on the Skakkebaek TDS hypothesis that the association between the number of beef meals eaten per week during pregnancy and sperm concentration in male offspring might be due to exposure to hormonal residues in beef. This hypothesis is plausible because during the years this cohort of fertile men were *in utero* (median year of birth was 1970), beef cattle in North America (where the majority of study participants were born) were routinely treated with the growth-promoting anabolic steroids. For example, the drug diethylstilbestrol (DES) was widely administered to beef cattle in the USA between 1954 and 1979 (Raun and Preston, 2002).

Although the use of hormones to promote an increase in lean muscle mass in beef cattle has not been legal in Europe since 1989, and while DES was banned in 1979, administration of combinations of other hormonally active drugs to beef cattle has continued to be a common practice in the USA and Canada. The International Joint Food and Agricultural Organization's World Health Organization Expert Committee on Food Additives (JECFA) published acceptable (tolerable) daily intake ADIs (TDIs) for all hormones in current use as growth promoters in beef cattle, although the assumptions used by JECFA to determine 'safe' daily human exposure levels in their 1988 report have been challenged (Andersson and Skakkebaek, 1999). There has been a trade dispute over the safety of hormone residues in beef going on for many years, with the European Union opposing importing hormone-treated beef from the USA and Canada. The position of the USA and Canada is that hormone residues in beef pose no threat to human health; this position is based primarily on research concerning the mutagenic activity of estradiol.

Over the past decade, there has been a shift in the focus of research in endocrinology, cancer and developmental biology based on findings that hormones, hormonally active drugs and

**In autumn 2006, the author served on the science advisory committee of the European Union regarding low-dose effects of xenobiotic estrogenic chemicals in food.*

environmental endocrine disrupting chemicals in food cause epigenetic modifications of DNA and histone proteins (methylation/acetylation), which result in 'programming' of genes during tissue differentiation. The consequence of exposure to xenobiotic estrogenic chemicals can thus be disruption of the programming of metabolic and reproductive processes via epigenetic changes in DNA and associated proteins rather than DNA mutations (Feil, 2006). In addition, initiation of cancer has been proposed to be due to epigenetic rather than mutational effects of xenobiotic chemicals (Feinberg *et al.*, 2006), and the relationship between red meat consumption and estrogen/progesterone receptor-positive invasive breast cancer in premenopausal women is thus also of concern (Cho *et al.*, 2006). Relevant to the issue of dose required to cause an effect is that epigenetic programming of genes is a normal developmental process that occurs at very low physiological levels of estradiol. For example, responses to estradiol in human cells in culture occur at and below 1 pM (0.28 pg/ml culture medium); this concentration *in vitro* is relevant to the endogenous biologically active (free) concentration of unbound and unconjugated estradiol in blood (Welshons *et al.*, 2006).

Whereas in adults acute exposure to very low doses of hormone residues in beef might transiently activate responses that cease when exposure ceases, during critical periods in tissue differentiation when epigenetic programming occurs, the consequences of exposure to a very low doses of xenobiotic estrogens and other hormonally active chemicals are typically permanent. For example, a very small increase of 0.1 pg/ml free serum estradiol in male mouse fetuses or a very low dose of a xenobiotic estrogen in neonatal male rats caused abnormal prostate morphology observable during development, as well as altered function and pre-cancerous lesions during later adulthood (vom Saal *et al.*, 1997; Timms *et al.*, 2005; Ho *et al.*, 2006). Exposure of pregnant female mice to a very low oral dose of ethinylestradiol (0.002 μg/kg/day) reduced testicular sperm production in male offspring (Thayer *et al.*, 2001), which provides experimental support for the hypothesis that estrogenic hormonal residues in beef might be a factor in the association reported by Swan *et al.*

There is thus an entirely different view today concerning the potential for harm from developmental exposure to very low doses of xenobiotics relative to the data available to JECFA based on research focusing on mutagenesis conducted in the 1970s and early 1980s (Andersson and Skakkebaek, 1999; Welshons *et al.*, 2006). This concern is elevated by the potential for synergistic interactions between the different combinations of hormonally active drugs in common use today; combinations of drugs with estrogenic, androgenic, progestegenic and glucocorticoid activity are typically used (Andersson and Skakkebaek, 1999). In addition, there is also the potential for interaction with other xenobiotic chemicals present in beef, such as pesticides and dioxin-like chemicals (Schecter *et al.*, 2001; Pang *et al.*, 2006) as well as chemicals used in plastic wrap and plastic food containers, such as bisphenol A (Welshons *et al.*, 2006). The focus on critical periods of vulnerability was a major factor in the Children's Health Initiative in the USA. This initiative was based on the realization that levels of xenobiotic chemicals that are safe for an adult can never be assumed to be safe for a fetus, infant or child; the maxim in pediatric medicine is that 'children are not little adults'.

The issue of vulnerability of children to exposure to xenobiotic estrogens and other hormonally active chemicals is central to the argument over the safety of low levels of hormone residues in beef, since the estimated acceptable (presumably safe) levels for human exposure to hormone residues in beef were established using daily sex hormone production values in children, in whom endogenous sex hormone levels are lowest. However, due to ethical considerations, information about daily production of sex hormones in children is extremely limited. Therefore, total daily production values in children were calculated using estimates of metabolic clearance rates obtained from studies with adults. Since children are not little adults in terms of physiological processes, the estimated reference values used to establish 'safe' exposure levels for hormone residues in beef may overestimate background levels in children by as much as 100–200-fold and thus dramatically underestimate risk (Andersson and Skakkebaek, 1999).

Attempting to relate events during fetal and/or neonatal life to adult disease or disruption of normal organ function is an exceedingly difficult challenge for epidemiology. This was tragically demonstrated by the fact that DES was administered to millions of pregnant women for almost 25 years before an association between exposure during fetal life and subsequent clear cell vaginal adenocarcinoma in female offspring was recognized by an astute physician. There is now also evidence for transgenerational transmission of this developmentally induced DES defect to subsequent unexposed generations (Ruden *et al.*, 2005).

Given the importance of this issue, the intriguing relationship reported by Swan *et al.* should serve to stimulate further research to investigate the basis for the association between maternal beef consumption during pregnancy and sperm concentration in male offspring. For example, a prediction based on the Skakkebaek TDS hypothesis is that if xenobiotics are causally involved, the finding of reduced semen quality should be the 'tip of the iceberg' and other reproductive pathologies should also be observed. Furthermore, women would also be expected to be affected by developmental exposure to xenobiotic hormones; studies relating maternal beef consumption to daughters' incidence of PCOS, age at adrenarch/menarche and post-natal growth rate would be predicted to show a significant relationship. On the basis of the shift in focus regarding the molecular mechanisms by which xenobiotic hormonally active chemicals can disrupt development at very low doses, and the advances in analytical techniques over the last 20 years, the risks associated with exposure during development to hormonal residues in beef should be revisited by JECFA and other regulatory bodies.

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