

GENERAL DISCUSSION

ROLE OF ENVIRONMENTAL FACTORS IN TIMING THE ONSET AND PROGRESSION OF PUBERTY

Chair: S. De Muinck-Keizer Schrama and A. Juul.

Dr R. Anderson (Edinburgh, UK)

We can only speculate at what stage of development the ovary may be susceptible to external influences because no hard data are available. We do not know if maternal smoking during pregnancy can influence germ cell survival in vivo in the developing fetus although an effect has been demonstrated in model systems in vitro. The possible effect of smoking on the age of menopause, and therefore the reproductive lifespan, is more amenable to study. There is evidence that smoking causes an earlier menopause but only if smoking occurs at the time of menopause. It is not related to a past history of smoking which may be due to compensatory mechanisms in the ovary regulating how fast follicles are activated and therefore effectively used up. There is much uncertainty in this field, and the fact that many germ cells are made and lost may suggest that a small influence might potentially have a large effect.

Dr S. Franks (London, UK)

Disorder of early follicle development in the ovary appears to be the key event in the aetiology of polycystic ovary syndrome (PCOS) but little is known about the cause of disordered follicle formation/development; the relationship with ovarian hypersecretion of androgens; and what genes are involved.

We still do not know what environmental factors cause the disordered folliculogenesis and disordered reproductive function in PCOS, and the intrauterine environment must be considered. Excess androgens in the maternal circulation are unlikely to have an effect because the placenta provides an effective barrier, but metabolic disturbance may have a role because we know that women with PCOS are more likely to develop gestational diabetes. We know nothing about the effects of environmental insults including endocrine-disturbing chemicals, although there is speculation that maternal smoking may have an effect on fetal ovarian development.

The postnatal environment may also be important especially at puberty which is the vulnerable period because of the activation of the hypothalamo-pituitary-ovarian axis. Changes in body fat distribution with central adiposity and insulin resistance are physiological in puberty and are amplified in patients with PCOS. Very little is known about the timing of puberty associated with PCOS and this is confounded by possible delay because of the alterations in the reproductive system.

G. Lyons (WWF, Godalming, UK)

Do the fetal ovaries in utero have aromatase? Environmental exposure to tributyltin, which is an aromatase inhibitor, may cause an increase in the androgen level.

Dr S. Franks

Transcripts of CYP19 (coding for aromatase) can be detected in human fetal ovarian tissue (Pezzi et al., 2003) but production of oestradiol is thought to be minimal or non-existent before birth.

Dr S. R. Ojeda (Beaverton, OR, USA)

I have no reason to believe that human and rat ovaries are different in the early stage of development. In the rat fetal ovary, there is a very strong increase in aromatase activity peaking at day 18-20 of gestation, and one can elicit this elevation via neurotransmitter-induced cAMP formation. The neurotransmitter vasoactive intestinal peptide is a strong inducer of aromatase activity and it is able to do so before the formation of follicles.

Dr S. Franks

There are not only similarities but also major differences in steroidogenesis and folliculogenesis between rodent and human ovaries. There are marked differences in the development of follicles which is virtually complete in utero in humans and rhesus monkey, and steroidogenesis is evident in fetal rodent ovaries but not in fetal human ovaries. There is also evidence that oestrogens influence the number of follicles in baboons.

Dr R. J. Aitken (Newcastle, Australia)

In the adult mouse ovary, the number of primordial follicles results from a dynamic interchange between the destruction of primordial follicles and the creation of new follicles from a stem cell population (Johnson et al., 2004). If a similar mechanism is present in humans there may be a possible environmental effect on the differentiation of a possible stem cell population.

Dr R. Anderson

There is no evidence for stem cells in the human adult ovary. The mouse experiment has not been confirmed by other laboratories and there are questions over the methodology used. There is no substantiated evidence of a renewable stem cell population in adult mouse.

Dr S. Franks

The dynamics of pre-antral follicular development is different from normal in PCOS. This may result in accumulation of follicles at the primary stage.

Dr A. Juul (Copenhagen, Denmark)

If exposure of the human fetal ovary to androgens can cause subsequent development of PCOS, patients with congenital adrenal hyperplasia (CAH) should have polycystic ovaries in the adult because the level of androgens is very high in the fetus.

Dr S. Franks

There are insufficient data to answer that question definitively, and these are confounding factors. Patients with classical and non-classical CAH have a high prevalence of polycystic ovaries which may be due to an androgenic factor in utero, although there may be other explanations. Maternal androgens from CAH patients are unlikely to affect the fetus because of binding by sex hormone binding globulin and metabolism by aromatase in the placenta. Mothers with androgen-secreting tumours during pregnancy do not usually have a virilizing effect on the fetus.

Dr M. E. Herman-Giddens (Pittsboro, NC, USA)

There are many theories and speculations advanced to explain the earlier puberty and menarche, and increased growth rate in children in the USA. There are genetic differences among the different racial and ethnic groups. African-American girls have increased insulin resistance which is possibly related to their typical developmental pattern of pubic hair growth preceding breast development (Herman-Giddens et al., 1997). Being overweight is associated with earlier puberty in girls. (Wattigney et al., 1999; Adair & Gordon-Larsen, 2001). For US children, a general increase in overweight and obesity has occurred due to excessive food intake, especially of fast, convenience 'junk' food, and lowered physical activity (Cavadini et al., 2000; Kimm et al., 2002; Ogden et al., 2002). In the livestock industry, steps are taken to maximize rapid weight gain, and it is economically desirable for the animals to enter puberty as quickly as possible. This is achieved in animals such as pigs and cattle by a complex dietary formula which is high in protein and calories, supplemented by restriction in movement. These conditions which are imposed on animals are similar to the modern lifestyle of US children. The increasingly poor quality of diet along with increased calories, also exposes the overweight children to more food hormones and endocrine disrupters. The growing exposure to environmental endocrine disrupters acting pre- and postnatally includes compounds such as insecticides, body care products and dental sealants which have a high concentration of phthalates, and other compounds with oestrogen-like activity. There are reports that infant soy-based formulas may affect the onset of puberty, although there is conflicting evidence for this (Setchell et al., 1998; Strom et al., 2001). Girls born small for gestational age may be prone to early puberty (Ibanez et al., 2000). Stress factors may be involved, and there is evidence that family break up with an absent father and possibly exposure to a new unrelated man in the household may result in early puberty in the children, especially in girls, although the explanation for this is unclear (Bogaert, 2005). The content of the diet, in addition to quantity, appears to influence development: a high vegetable and fibre content especially at the pre-school age causes a delay in the onset of puberty. (Berkey et al., 2000). Cultural changes with a trend towards hypersexualization may also have a role to play.

The trend to earlier puberty is a multicausal effect, and there is a cumulative effect of many factors acting especially on individual girls but also on the whole population in general.

There are still many questions to be answered such as whether there is an optimal (healthiest) age for puberty and if earlier puberty can affect reproductive health. Practical biomarkers of pubertal events must be developed, and we need consensus regarding the most accurate methods for assessing the onset of puberty, and the medical implications of the changing age of pubertal

development. The study of the effects of environmental exposures, including multiple and cumulative exposures, must continue, and different countries should assess secular trends on a regular basis and respond accordingly.

Dr F. Biro (Cincinnati, OH, USA)

The parameters 'age of menarche' and 'age of onset of puberty' are often used interchangeably, and it is generally assumed that both are closely related. However, it appears that the correlation coefficient between the two has been declining over the past six decades. The reported variations in age of sexual maturation may be partly explained by comparing age of menarche in some studies with age of onset of puberty in others, although the age of both parameters appear to be declining in girls. It appears that although there are factors in common for both, increasingly the factors involved in age of menarche are different from the factors associated with age of onset of puberty. We are still unclear about the nature of these different factors, although nutrition, health conditions and endocrine disruptors may all contribute (Biro et al., 2006).

Dr T. Schettler (Newburyport, MA, USA)

This Workshop has discussed syndromes consisting of constellations of different features which are not always aggregated in the same way. For example, in sexual maturation we consider an aggregate of events including obesity, thelarche, onset of puberty and onset of menses, but each of these may be influenced independently by different factors. We must keep in mind that syndromes are an aggregate of features resulting from complex interactions within a causal web.

Dr J.-P. Bourguignon (Liege, Belgium)

This is unpublished data (Hauspie, private information) giving an update of pubertal development in several thousand children from the Flemish community of Belgium. Data are available at <http://www.vub.ac.be/groecurven>. A total of 17 875 children were split into three birth cohorts and studied at 0-3 years of age (3567), 3-18 years of age (13 506) and >18 years of age (802), and examined by physicians.

Male puberty

Half of the boys (P50) reached testicular stage T4 (4 mL volume) and genital Tanner stage G2 by 11.5 years. However, 3% (third centile) reached T4 as early as 7.5 years giving a skewed distribution towards the lower age. The asymmetrical distribution emphasizes that not only the mean age at a given pubertal stage, but also the distribution at that stage is important. At later stages, T12 (testis volume 12 mL) was only reached by 97% of the boys by the age of 18 years, and the 97th centile for 18-year-old boys did not include T15 (testis volume 15 mL) or Tanner stage G5 indicating asymmetry of distribution with an inverse skew towards the older age group. These results indicate that the mean age is not changing but the distribution is changing.

Female puberty

In the Flemish girls, P50 for age of menarche and the age distribution have not changed (www.vub.ac.be/groecurven). There is no change in the mean ages of the different stages of breast development but the earlier stages of breast development are now being identified in some

younger girls with breast stage B2 being reached by 3% of the girls at the age of 7.6-7.7 years. This earlier breast development in Belgium is similar to the US findings. The age range of breast development has increased in comparison with the previous results of 1985, and then an increased range of ages of pubic hair developmental stages. A study in France published several years ago (Clavel-Chapelon, 2002) comparing 1920 and 1950 birth cohorts demonstrated an inverse relationship between age of menarche, which was decreasing, and age of achieving regular menstrual cycles, which was increasing. The 1920 cohort had less than 10% of individuals failing to achieve regular cycles within 5 years of menarche, whereas 20% of the 1950 cohort still had not achieved regular cycles 5 years after menarche.

We should revise our methods of analysing puberty, and more subtle changes should be recorded rather than just the mean age at which a given sign is identified.

Dr F. E. von Eyben (Odense, Denmark)

There are differences in sexual development between USA and Europe. The relationship between body mass index (BMI) and puberty is similar in USA and Europe, but there is greater obesity in the USA. However, it has been suggested by specialists in obesity that Europeans follow US developments after a 20-30 year delay. This rather pessimistic perspective may indicate that the European trends in menarche and puberty will 'copy' the USA experience if we adopt their lifestyle and exposure to environmental factors.

Dr A. Juul

Your comments are more 'realistic' than 'pessimistic'. A recent paper in the Danish Medical Journal indicated that obesity is increasing in Denmark (Pearson et al., 2005). The BMI for 6-9 year old children is similar in Denmark and USA indicating that factors other than BMI are causing the differences in sexual development between USA and Europe.

Dr F. Biro

Body mass index is an index of ponderosity rather than adiposity, and reflects fat mass as well as fat-free mass. The composition of BMI may reflect a higher proportion of body fat in US children when compared with European children, although there are no studies that compare this, to my knowledge. There has been a trend in the USA to become so-called 'couch potatoes'. Kimm et al. (2002) indicated that the majority of female teenagers in USA do not participate in any form of regular physical activity and two-thirds of 16-year-old girls have no regular physical activity. The potentially greater proportion of lean body mass in BMI of Europeans may become converted to fat if Europeans adopt the less active lifestyle of US youth.

Dr A. Juul

The age at voice break has particular relevance to choir boys, and we measured voice break in relation to BMI discovering that boys in the upper quartile of BMI had voice break at an earlier age (Juul et al., unpublished data). We assume that voice break is an indirect measure of puberty indicating conflicting results with the US data where slim boys tend to have an earlier puberty

compared with fat boys. There must be other factors which might explain why obesity appears to delay puberty in the USA but advances the age of voice break in Denmark.

Dr J.-P. Bourguignon

We must remember that puberty itself causes an increase in BMI making an interpretation of causality rather difficult. The standard deviation score is based on the mean score and is not a useful marker for early maturation.

Dr S. Ojeda

There are difficult problems in trying to discover new genes which regulate puberty including defining a marker for puberty. We need to find genes which initiate the process using non-invasive technology. We cannot study activation of genes in the hypothalamus or pituitary, but they would be accessible if they were also activated in skin. This would enable us to design tests to discover how environmental endocrine disruptors could affect the gene clusters involved in the regulation of puberty. No such genes have been identified with certainty although the Cut-like 1 gene is a possible candidate. Cut-like 1 is present in most tissues and controls different processes. Male mice lacking Cut-like 1 exhibit reproductive phenotypic abnormalities including infertility. It would be interesting if Cut-like 1 expression was controlled by oestradiol and/or other steroid hormones. In such a case, activation of this gene could be assessed in buccal mucosa or skin keratinocytes and may be a marker for gene activation associated with puberty.

Dr W. G. Sippell

Tumour suppressor genes are upregulated at the beginning of puberty.

Dr S. Ojeda

There are two aspects to that observation. First, there is a known association between sex development at puberty and an increased incidence of cancers such as testicular germ cell tumours. Secondly, we now know that many so-called 'tumour suppressor genes' are involved in normal processes. The name assigned to genes is determined by the first described function, and this is why the KISS 1 gene was first recognized to be associated with suppression of metastases from malignant tumours, but we now know that it has a key function in the regulation of luteinizing hormone and follicle-stimulating hormone secretion from the pituitary. Similarly, fibroblast growth factor causes mitogenesis in fibroblasts but is also involved in the development of brain and many other tissues. The generic term 'tumour suppressor gene' is largely a misnomer.

Dr W. G. Sippell

Many paediatric immune disorders such as rheumatoid disease, asthma and allergies disappear at puberty. Are the 'tumour suppressor genes' which are active at puberty also helping these disorders?

Dr S. Ojeda

This is a very interesting finding discovered by the use of microarrays and quantitative proteomics, but not yet explored in detail. There are a number of genes, which are involved in immunological

processes, that also show activation during puberty but we do not know why. For instance, the gene whose expression increases most prominently in the hypothalamus of male monkeys at puberty is a gene in the tumour necrosis factor pathway, which is a surprising finding.

Dr J.-P. Bourguignon

We must realize the problems associated with species differences when trying to find an animal model for human puberty because some primates are the only other animals which have the unique hiatus between birth and sexual development. As a paediatric endocrinologist I am very impressed by all the data on fish and other animal species, and we need further comparative studies in order to correlate human experience with experimental data.

Dr E. den Hond (Mol, Belgium)

There is a problem of selecting the correct biomarkers in epidemiological research when, for example, assessing oestrogens and anti-oestrogens, and androgens and antiandrogens. We must also know when is the correct time to make measurements because effects are different prenatally and postnatally, and pre-pubertally and post-pubertally. Researchers should pursue similar lines because if we all look at something different we may achieve nothing at the end.

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