Institute for Environment and Health

MALE REPRODUCTIVE HEALTH RESEARCH PROGRAMME: FIRST REVIEW MEETING, OCTOBER 1999

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1 General introduction

1.1 BACKGROUND

In recent years, results from several scientific studies have indicated temporal and regional changes in the reproductive health of humans and wildlife. In humans, effects reported have included increased incidences of testicular and breast cancer, birth defects and, in some regions, declining sperm count and quality. Experimentally, several naturally occurring and anthropogenic chemicals have been found to possess the potential to interfere with various aspects of the endocrine system, one consequence of which is the alteration of reproductive functions. These findings have led to concerns that chemicals present in the environment may be contributing to the deleterious effects on human health and wildlife that have been observed.

The Department of the Environment, Transport, and the Regions (DETR) leads an inter-departmental group established to coordinate research in the UK into the possible effects of endocrine disrupting chemicals (EDCs). Recently, a report has been produced on the work of the group.[1] To date, this group has considered many topics, for example the potential effects on wildlife (such as fish), the marine environment and human reproductive health, and the estimation of human dietary intake of suspected EDCs.

Following publication of the assessment by the MRC Institute for Environment and Health (IEH) on environmental oestrogens,[2] the inter-departmental group identified human male reproductive health as a priority for further investigation. On the basis of a number of international reports and meetings, it appears that the evidence for an increase in testicular cancer rates in developed countries is strong but the evidence for other effects, such as alterations in semen quality, is less certain and subject to considerable geographical variation.

A workshop sponsored by the Health and Safety Executive (HSE) and held at IEH in November 1996 reviewed the available information on male reproductive health and identified a number of research topics requiring further investigation involving studies on trends in male reproductive health and the possible influence of occupational and environmental factors. In particular, four questions were posed by the workshop:

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- Is male reproductive health in the UK changing? Endpoints identified for possible consideration included: semen quality; time to pregnancy (in so far as it reflects male reproductive health); and the incidences of testicular cancer, prostate cancer, hypospadias (a deformity of the penis) and cryptorchidism (undescended testes).
- Do occupational or environmental exposures to chemicals directly influence male reproductive health?
- Do maternal (or paternal) exposures to EDCs affect the reproductive health of male offspring?
- What effects do other factors (e.g. lifestyle, diet, temperature, area of residence) have on trends in male reproductive health?

In order to address these questions, a research programme was established as part of the wider activities of the interdepartmental group on endocrine disrupters.

1.2 SYNOPSIS OF FUNDED STUDIES

In total, four projects were selected for funding by the UK government at a cost of £1.7 million over three years, from August 1998. These were funded by a consortium comprising HSE, DETR, and the Department of Health (DH). Soon after the UK government call for proposals, the European Chemical Industry Council (CEFIC) put out a similar call for research, and one of the projects discussed here (Cherry *et al*., Section 2.1) attracted joint funding. A fifth project (Toledano *et al*., Section 2.5) is being carried out separately at the government-funded Small Area Health Statistics Unit (SAHSU), but its aims fit the general portfolio of the UK research programme. The five projects are summarised below.

UK multicentre study of occupational and environmental exposure to chemicals and male fertility (University of Manchester and University of Sheffield)

This is a case–control study involving up to 6000 men attending any of ten infertility clinics over a 2-year period. It is investigating whether the distribution of occupations or inferred exposures to chemicals differs between infertile men (cases) and men attending the same clinics but with normal sperm analysis (controls).

Historically prospective cohort study of Scottish male reproductive health (MRC Reproductive Biology Unit, University of Edinburgh)

This study is attempting to distinguish the effects of parental exposures (intrauterine and perinatal) and direct effects (adult exposure and lifestyle) using a novel matched pairs design to examine twin births. The study will also provide baseline data on sperm quality in a cohort (random sample) of young Scottish-born men.

Environmental risk factors for hypospadias: a population-based case–control study in three health regions (Imperial College, London)

This is a case–control study of hypospadias in the three regions. Approximately 200 cases and 200 controls will be investigated to explore the influence of maternal and paternal occupational and environmental exposures and lifestyle factors.

An assessment and analysis of existing surveillance data on hypospadias in UK and Europe (London School of Hygiene and Tropical Medicine)

This is an analysis of UK and European databases on hypospadias to look for trends in relation to time, geographical location and maternal occupation.

Geographical epidemiology of prostate and testicular cancer in Great Britain (SAHSU, Imperial College, London)

SAHSU, based at Imperial College, London, is studying the geographical epidemiology of testicular cancer and cryptorchidism in a project funded by DETR, DH, HSE, the Scottish Executive, the National Assembly for Wales, and the Northern Ireland Office.

1.3 THE REVIEW MEETING AND REPORT

To facilitate communication between researchers, funders and the stakeholders involved in the research programme, it was agreed to hold annual meetings to review progress in the portfolio of projects. This report summarises the presentations and discussions that occurred at the first research review meeting held at IEH in October 1999. The programme for the review meeting is presented in Annex 1. Participants included members of each of the project teams, representatives from the funding departments and from CEFIC and environmental groups, as well as independent scientists working in the field (see Annex 2). The principle aims of the meeting were:

- to discuss the progress of each project with stakeholders; and
- to allow all participants in the programme to become familiar with each others' work.

Information on the individual projects is presented in Section 2 of the report, and includes the abstract describing the project (submitted by the research team before the meeting), and a summary of the progress on each project to date, based on the presentation made by the research team and the associated discussions during the course of the review meeting. Section 3 summarises the more general discussions on questions of male reproductive health that took place at the end of the meeting.

1.4 REFERENCES

1. Governmental Interdepartmental Group on Endocrine Disrupters (1999) *Report of Activities Between November 1995 and May 1999* (Ref No 99EPO452), London, UK, Department of the Environment, Transport and the Regions

2. IEH (1995) *IEH Assessment on Environmental Oestrogens: Consequences to Human Health and Wildlife* (Assessment A1), Leicester, UK, Institute for Environment and Health

2 Individual study reports

2.1 UK MULTI-CENTRE STUDY OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE TO CHEMICALS AND MALE FERTILITY

Principal investigators - Professor Nicola Cherry,[1] Professor Harry Moore,[2] Dr Roseanne McNamee,[1] Dr Andrew Povey,[1] Dr Allan Pacey,[2] Dr Gary Burgess[1]

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2.1.1 Abstract

Introduction

Previous studies on the effects of male fertility have been carried out using volunteers exposed at work, by studying pregnancy rate, or by examining the occupations and exposures of couples declaring themselves to be infertile by attending a fertility clinic. The great strengths of the latter approach are that:

- a problem with fertility (rather than simply a change in sperm quality) has been demonstrated;
- all couples are sexually active and attempting to conceive, hence contraception is not an issue;
- semen analysis can be obtained for most males;
- motivation to participate is very high; and
- an appropriate comparison group is readily available, that is men attending a fertility clinic that have normal sperm constitute a referent group drawn from the same base population as 'cases' (men with abnormal semen analysis).

This approach also has considerable potential for identifying occupational exposures not well documented to affect fertility and it can be easily extended to include non-occupational

exposures. In addition, the total impact of occupational exposures on fertility in a population group can be addressed. However, a weakness is that it will exclude men with chemically induced impotence or lack of libido, and there are also potential methodological problems: (i) the difficulties of obtaining appropriate measures of exposure across a population in a wide diversity of occupations; (ii) the large numbers needed to achieve adequate power; and hence the problems of (iii) measuring sperm parameters reliably across centres; and (iv) measuring appropriate markers of exposure economically.

Rationale for the present study

The present study is designed to investigate which occupational exposures are risk factors for male infertility. It specifically addresses the risk associated with exposure to individual solvents and to individual heavy metals, as measured in biological fluids, and it also seeks to identify new substances (occupational and non-occupational) not previously linked with infertility. It takes advantage of all the positive features of the fertility clinic approach while addressing each of the potential problems associated with it. Sperm parameters are measured centrally where possible, using computer aided semen analysis (CASA), and strict quality control procedures are implemented. Exposure assessment has a 'two-stage' design with assessment at the second, more accurate but expensive stage carried out on subgroups of men selected so as to optimise the cost-efficiency of the study, while still retaining high statistical power. The assessments of exposure and of fertility are carried out independently by different organisations (Manchester and Sheffield), so reducing the possibility of bias. Finally a data archive, consisting of biological samples and CASA derived parameters of motility and morphology, is being retained to enable future hypotheses about exposures or mechanisms to be tested.

Study objectives

The primary objective of this study is to determine whether risk of infertility in men is related to occupational exposure to chemicals. Specific aims are to determine, for male patients newly attending for fertility investigations in ten UK cities:

- whether the distributions of occupations or inferred exposures to chemicals differ between infertile men (cases) and men attending the same clinics but with normal semen analysis (referents); and
- whether concentrations of organic solvents and heavy metals in blood, urine and seminal plasma differ between cases and referents.

The second objective is to investigate the relationship between infertility and non-chemical occupational factors and other non-occupational factors, which should be considered as potential confounders in this and future studies. Specific aims are to compare:

- the distributions of inferred, non-chemical occupational exposures (e.g. heat, vibration) between cases and referents; and
- the distributions of cotinine in urine and seminal plasma, declared alcohol intake, age, previous illness, medications and wearing of constrictive clothing, between cases and referents.

A third objective is to provide unbiased comparisons of the distribution of sperm parameters between geographic centres in the UK. Specifically, the aim is to determine, among a subgroup of men whose partners are diagnosed as having total tubular occlusion, if there are regional differences in sperm parameters and whether these differences can be explained by risk factors identified under the objectives described above.

A final objective is to investigate the effects on male fertility of maternal and neonatal exposures to substances thought to be endocrine disrupters.

Study design

Selection and recruitment of subjects

Subjects are drawn from male patients newly attending any of ten hospitals in the UK for fertility investigations. Subjects may be recruited through a specialist infertility clinic, a gynaecology clinic, or the hospital laboratory. To be eligible for recruitment subjects must be aged 18 years or more, have been having unprotected intercourse for 12 months or longer, and not have had a semen test (or results of a test) prior to recruitment.

Identification of eligible patients is carried out by a research nurse appointed in each of the hospitals, with assistance from administrative or laboratory staff as required. Identification is achieved by review of clinic or laboratory appointment lists and referral letters, and, in some centres, by direct contact with patients themselves.

Recruitment procedures have been tailored to fit the referral patterns at each hospital, and once eligible subjects are identified there are two main methods used, either the patient is approached at the clinic or laboratory following referral to the clinic, or is approached at the laboratory at the time of a routine semen test prior to referral.

Questionnaires and interviews

Four questionnaires are completed by each subject. A 'Work and Lifestyle' questionnaire is filled in by the subject before the research interview. More detailed information on exposures is collected at the interview, concentrating on jobs held during the preceding 24 months. If any of 116 job titles is encountered, this identifies the need for one of 30 specialist job questionnaires designed for the study. At the end of the interview the nurse hands out questionnaires relating to diet and maternal factors, which the patient completes at home and returns by post.

Collection and processing of biological samples

At the interview the nurse also collects blood, urine and semen samples which are produced on site. The samples are then passed immediately to the laboratory. Standard protocols for the collection and processing of samples have been implemented in all hospitals. Sperm motility is video recorded by lab staff using a computer-controlled outstation. The same equipment (with the same configurations) is used in all hospitals. Ten semen smears are prepared according to World Health Organization (WHO) guidelines and fixed in ether/ethanol for morphology analysis. Sperm concentration is determined manually following WHO guidelines.

Sperm analysis

Sperm analysis takes place centrally, in Sheffield. Computerised analysis of sperm motility is undertaken from local video recordings using the IVOS system (Hamilton-Thorne, Mass,

USA) and the Hobson Sperm Tracker. Semen smears from the hospital samples are stained using the modified Papanicolaou staining procedure. Sperm morphology is then analysed by computer, using the Hobson Sperm Tracker and Hamilton-Thorne morphology equipment.

Definition of cases

Cases are defined as patients who are judged to be infertile on the basis of sperm parameters, that is those men who have less than 12 x 10^6/ml progressively motile sperm in their initial semen sample. Referents are defined as all other patients. A second case–referent analysis will define cases on the basis of the current WHO reference value for morphology, that is less than 30% normal sperm morphology, with all other men defined as referents. Classification as a case or referent is blind to exposure. With few exceptions (e.g. patients found from questionnaire to have chromosomal or congenital abnormalities, or those treated with chemotherapy), all men recruited to the study are eligible for inclusion as a case or a referent.

Measurement of exposure

Chemical exposures affecting the processes of spermatogenesis and sperm maturation are specifically targeted. Exposure assessment follows a two-stage method. At the first stage, questionnaire data are rated for exposure by a panel of three occupational hygienists, blind to case status. This has two purposes, to generate hypotheses about substances not previously suspected of causing infertility, and to determine which subjects will be selected for the second stage of assessment.

The second stage of assessment examines exposure only to individual organic solvents and heavy metals. For those men selected for investigation at Stage 2, the nurse arranges a special visit to the clinic at the end of a working day for further samples of blood, semen and urine to be collected. Markers of internal dose are measured in these samples by analysis at the Health and Safety Laboratory (HSL) in Sheffield. Aliquots of all blood, urine and seminal plasma samples and sperm pellets are archived for future studies.

Information on possible exposures to endocrine disrupters *in utero* through maternal employment or diet, and currently to phytoestrogens, are assessed by questionnaire. Biological samples are stored in glass containers at -70 ºC to permit later assessment of concentrations of specified endocrine disrupters should this appear justified.

Measurement of confounders

The job history questionnaire, sent to all men in advance of the research interview, also addresses current health and medication, fertility history in this and previous relationships, childhood illnesses, hobbies, whether constrictive clothing is worn, and current alcohol and cigarette consumption. Tobacco use is also monitored by measuring cotinine in urine and seminal plasma.

Project management and quality control (QC)

Under the supervision of the principal investigators, two full-time coordinators have been appointed. Ms Julie-Ann Clyma, based in Manchester, is responsible for study design, recruitment of subjects, exposure assessment and QC in these areas, as well as overseeing the work of the nurse interviewers. Dr Helen Baillie, based in Sheffield, is responsible for all aspects (local and central) of semen sample preparation and analysis, and for quality control of sperm parameter data.

QC procedures for recruitment

A procedures manual has been drawn up for each centre. In order to check adherence to procedures, each research nurse must complete standardised audit forms to document activities. All nurses attended a training session in advance of starting recruitment, to learn interview techniques and to become familiar with protocols. Regular site visits are made to inspect procedures.

QC procedures for sperm parameter data

There has been criticism that temporal and geographical differences in human semen quality might be due in part to inadequate quality control, inter-centre assay variation and statistical bias. Therefore, a crucial aspect of this study is to ensure complete comparability between centres for the assessment of semen quality (sperm concentration, motility and morphology), with strict quality control of all assays.

Comprehensive QC procedures are currently in place to monitor all aspects of the semen analysis protocol. All andrology technicians directly involved in semen analysis attended a

two-day training workshop at Sheffield prior to the project, to learn techniques and to become familiar with protocols. Regular site visits are made to inspect procedures.

QC procedures for biological monitoring of exposure

Each centre is provided with a standard sample collection pack and staff have been trained to ensure that the biological samples are handled and processed appropriately. HSL laboratories routinely analyse biological samples for the chemicals identified in this proposal. HSL has relevant accreditation (e.g. ISO 9001 and IiP) and has well established QC mechanisms. In Manchester, procedures have been developed for the routine analysis of cotinine in biological samples in the newly established exposure laboratory. Appropriate QC schemes, both internal and external, will be applied.

Current progress

Recruitment of centres has been staggered, with the first starting in January 1999 and the most recent in September 1999.

2.1.2 Meeting presentation and discussions

Initiation of the project

The project received Multi-Regional Ethics Committee (MREC) approval one year ago, and centres are still entering the study. Nine centres are active in the project and three more have agreed in principle to participate. Thus, there will be two more centres than the ten originally planned.

Difficulties were experienced initially in some clinics, with nursing staff appointed for the study being allocated to general clinic duties if there was a shortage of staff. This has been resolved by providing funding for nurses only if the centre involved can undertake that the nurse will be dedicated to the project. Conference calls among the nurses and annual meetings help to keep them involved and informed as part of a team.

The first set of biological samples is ready for analysis, and questionnaires from the first 250 cases have now been subjected to preliminary analyses.

Recruitment and participation

Recruitment is increasing with each succeeding month, though not as rapidly as initially hoped. Steps are now being taken to improve recruitment. One major impediment to recruitment has been the requirement that subjects must not have had any previous semen analysis. General practitioners (GPs) are now being asked, contrary to usual practice, not to seek semen analysis before potential subjects are referred to a participating clinic; other subjects are being recruited when they present at the clinic for their first semen analysis. Of those who arrive for their first appointment, 80% agree to participate in the study.

Semen analysis

The need to minimise variation in sample collection and analysis, both over time and between centres, is met by using standard protocols for local sample collection and processing by means of computer outstations with predefined automated procedures. Additional funding may be needed to purchase two additional outstations for the two extra centres involved in the study. Sperm parameters are recorded locally on video and then centrally processed and analysed. Each of the centres is a member of the National External Quality Assurance Scheme, and personnel training is undertaken at each centre.

Several methods are used to validate semen analysis. Two commercial CASA machines are used, each working by a different method, and manual counting and fluorescence activated cell sorting are also used to validate sperm counts.

The cut-off point to assign patients as fertile or infertile is based on WHO criteria. The cut point is reasonably sensitive and, if considered to be on a J-shaped curve, occurs just as the curve begins to rise. In this study, all participants are assigned as either a case or a control, based on the defined cut point. There might be justification for selecting as controls only those with a definitely high sperm count. It is recognised that all measured sperm parameters are subject to within-individual variation. Days of abstinence before sampling, for example, are known to affect the count, and such factors, which may confound results, are being recorded.

Of the first set of subjects, 41% met the criteria for infertility, which fits well with the starting assumption that 45–55% of subjects would prove to be cases.

Preliminary analysis of questionnaires

Based on an analysis of the first 250 subjects, 42% were classified as office workers, 50% as manual workers and 8% as unemployed. The occupation recorded is that held over the past two years. Depending on employment history, specialist questionnaires may be used. For example, 69 specialist questionnaires on workers exposed to lead, 11 on painters, eight on agricultural workers and five on welders have been used in the first 250 cases. Based on preliminary questionnaire findings, 8% of cases were exposed to solvents, 10% were occupationally exposed to metals, 10% to physical agents and 2–3% to pesticides. These preliminary findings confirm the expected power of the study to identify differences in occupations between infertile and fertile men.

Preliminary information from questionnaires on maternal exposure while case subjects were *in utero* and on early childhood events has revealed that 39% of mothers were working at the time of conception. While 3% of mothers were vegetarians at some time, only 1.5% were vegetarian at the time of conception. Of the cases, 37% were breast fed as infants, 3% were fed soya milk, and 8% had had a genital anomaly corrected by surgery as a baby.[a]

Chemical analysis

At the outset, and to test prior hypotheses, biological samples are analysed for organic solvents and heavy metals. Chemicals are analysed in blood, and any metabolites are analysed in urine and seminal fluid. The European Chemical Industry Council (CEFIC) has been asked to assist in providing information on which particular endocrine disrupting chemicals (EDCs) should be analysed. Other lists of potential EDCs may also be considered, as appropriate. Analysis for chemicals other than solvents and heavy metals (including possible endocrine disrupters) will be conducted towards the end of the project.

[a] The classification 'surgical correction of a genital abnormality' did not generally include circumcision, although it was probably counted if the procedure was conducted in early infancy for medical reasons.

2.2 HISTORICALLY PROSPECTIVE COHORT STUDY OF SCOTTISH MALE REPRODUCTIVE HEALTH

Principal investigators - Dr Stewart Irvine,[1] Pamela Warner,[2] Dr Richard Sharpe,[1] Dr Raymond Agius[2]

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2.2.1 Abstract

Introduction

Background

A substantial body of evidence has accumulated in recent years suggesting that human male reproductive health may be deteriorating. There have also been reports of alligators with abnormal male genital development and of reproductive changes in fish and birds. At the same time, there have been controversial reports of adverse changes in human semen quality, alongside reports of an increasing incidence of congenital malformations of the male genital tract, such as cryptorchidism and hypospadias, and of an increasing incidence of testicular cancer. Unfortunately the evidence remains worrying, but inconclusive. It has been suggested that these changes may be due to environmental xenoestrogens acting during development. Although there is now a large quantity of data indicating that this is a plausible hypothesis, evidence of causality, rather than association, remains to be provided. This is now a large and complex field. Moreover, it is clear that many other factors, including lifestyle factors such as smoking, dress habits and even time spent commuting, may be relevant. There are few data on the implications of these changes for the public health. National or regional differences in semen quality may have a different aetiology than secular changes. Hence, national differences may have little effect on the achievement of pregnancy, for the vast majority of the population. On the other hand, small secular shifts in semen quality, if resulting from exposure to environmental toxicants, may be prejudicial to fertility, to the health of offspring, and to health generally. In particular, such exposure may be evident in specific dimensions of sperm quality, such as function.

Problems with existing data

The existing data on male reproductive health present a number of shortcomings. Remarkably, while there are nationally collected data on testicular cancer and on male genital tract malformations, there are no comparable data sets relating to semen quality. For this reason, the present study is focused on this endpoint. Unfortunately, existing studies on semen quality and its determinants can be criticised on a number of grounds.

Subject selection bias has been a major problem. Many studies have focused on particular groups of men, examples including candidate semen donors, men storing sperm prior to vasectomy, and the male partners of infertile couples, who may well be unrepresentative of the population, limiting the generalisability of the results.

Semen analysis methodology has also been criticised as being poorly standardised, and not subject to rigorous external quality control.

Relationships between the conventional criteria of semen quality and biological fertility are poor, except at extreme values. Even where fairly substantial regional differences are found in population means of sperm quality, the impact on fertility outcomes, such as time to pregnancy, are small.

It is acknowledged that data relating to any decline in sperm quality are equivocal. Even if such a decline could be demonstrated, the important research question, causation, remains to be answered. If environmental exposures to toxicants and/or endocrine disrupters have adverse effects on semen quality, then the true pattern of decline will depend on trends in industry and agriculture, in distribution of the workforce across occupational categories, and domestic exposures. Rapid secular changes in these, and the impact of protective legislation and media campaigns, mean that trends in sperm quality over the past ten years may be uninformative about trends over the next decade, and hence of little use in protecting the health of future generations. What is required is evidence as to the specific exposures prejudicial to male reproductive health.

While it is hypothesised that exposure to environmental toxicants has adverse effects on the reproductive health of males, there is less information on the relative importance of the timing of exposure: perinatal versus adult. If there has been a decline in the sperm quality of adult

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males this may be a result of perinatal exposures, themselves related to parental exposures, perhaps to chemicals that are no longer in widespread use in the UK. Secular changes in sperm quality may be partly or entirely due to direct exposures (of the adult male) to environmental toxicants and, if so, the effect may be reversible. Certainly, this aetiology offers more scope for an intervention to reduce exposure, to the immediate benefit of the reproductive health of the current generation of adult males.

Studies looking at intra-uterine exposures are complicated by the 20-year time lag between exposure and measurement of effect (semen quality assessment). The study of intra-uterine exposures has as a potential confounder the domestic milieu of the family, nutrition and other general health factors. Therefore, a pragmatic way to begin to address this question would be to control for intra-uterine effects and familial effects, by studying pairs of brothers matched for virtually all fetal and familial factors (twins) and comparing them to non-twin brothers (where fetal factors, infant feeding and secular trends in background exposure may differ, though familial factors will be very similar).

Study objectives

The main objectives of the study are described below.

- To obtain a 1999–2000 estimate of (a) exposures to various factors suspected to adversely affect sperm quality and (b) sperm quality in a cohort (random sample) of young Scottish-born men, and of any association between these.
- To distinguish the effects of parental exposures (intra-uterine and perinatal effects) and direct effects (adult exposures and lifestyle), using a matched pairs design to study twin births (who share the same uterine exposure to endocrine milieu and toxicants, and very similar early childhood nutrition).
- To provide methodological insight to the measurement of sperm quality in normal men (quantitative measurement as opposed to binary infertility case diagnosis). This will inform the interpretation of findings in the present study but also, importantly, provides the detailed knowledge required to optimise design of future studies into male reproductive health.

Study design

The design is that of an historically prospective cohort. The cohort will comprise a random sample of men, born in Scotland between 1967 and 1977 (aged 20 to 30 in 1997) and currently residing in mainland Britain. They will be contacted by mail, and the research design comprises three stages as described below.

Three-stage research design

Stage 1 A postal survey

The postal survey comprises the following assessments:

- questionnaire completed by the man;
- if his mother is alive, and if he is willing to forward it, a brief questionnaire completed by her; and
- if willing, a semen sample provided by him at home and mailed in.

The target is 800 men, each with questionnaire and semen quality data, plus a questionnaire completed by the mother. The semen quality data will be analysed in relation to questionnaire responses. This would provide a 1999–2000 estimate of semen quality in a random sample of young Scottish-born men, of their exposures to various factors suspected to adversely affect sperm quality, and of any association between these.

Stage 2 A methodological sub-study of the reliability of sperm quality assessment in normal men, and of short-term factors affecting variability in semen quality

This sub-study would be achieved by asking a sample of the men participating in Stage 1 if, after an interval of 6 months, they would provide a repeat semen sample and complete a further very brief questionnaire on any change in health or lifestyle.

The target would be for 200 men to supply repeat samples and questionnaire data. Change in sperm quality would be analysed in relation to season and to changes in lifestyle, health or exposure. This will provide methodological insight into the measurement of sperm quality in normal men.

Stage 3 A detailed matched-pairs study involving three groups of 100 pairs: twins, non-twin brother pairs and non-sibling matched pairs

The male twin pairs would be in the same age range and selected in the same way as Stage 1, though targeted to twin births. The brother pairs would comprise 100 men already in the survey, plus their brothers. The non-sibling pairs would be obtained by matching 100 or more pairs from the remaining 700 men in Stage 1. Within pair differences in sperm quality would be analysed in relation to degree of matching (twin, sibling or non-sibling) and to within-pair differences in adult exposures, birth order/spacing, infant feeding, parental/uterine exposures. Between pair variation in sperm quality would be analysed in relation to maternal factors. Since this part of the study would be strengthened by comprehensive assessment of environmental exposures and lifestyle factors, particularly those that may have direct effects on semen quality, it is intended to undertake an assessment by a trained interviewer.

Matched pairs is a powerful study design for elucidating effects. By utilising the phenomenon of twin births (who share the same uterine exposure to endocrine milieu and toxicants, and very similar early childhood nutrition), this design provides the opportunity to distinguish the effects of parental exposures (intra-uterine and perinatal effects) and direct effects (adult exposures and lifestyle). The more detailed assessment of adult exposures in the twin pairs allows much more rigorous examination of exposures with potential to affect sperm quality, as well as validation of the questionnaire assessment of exposures used in Stage 1 and 3. These three groups of matched pairs will allow delineation of the relative contribution of genetic, familial, uterine and direct effects on male reproductive health.

Subject identification and recruitment

The population sample has been obtained through the Registrar General for Scotland's Office which can provide NHS number and details of the Health Board with which the man is currently registered. Contact with the subject then requires a cascade approach:

- letter to Health Board, enclosing a letter to forward to subject's GP;
- letter to GP enclosing a letter to the subject, for forwarding by the GP if deemed appropriate; and

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- letter to the subject describing the research and its aims, asking for his participation, and enclosing a reply slip and freepost envelope, to enable him to indicate willingness to participate, and if prepared to participate, his address.

For the main survey, males born on two or three particular days of every month of every year have been extracted, ensuring a cohort of approximately 10 000 men, to achieve complete data for 800. Individuals selected by the search are matched by NHS number to current records, and identified as dead, matched or unmatched. For matched records current Health Board registration can be supplied. For unmatched records hand matching is undertaken. The sample has been confined to men born in Scotland and currently residing in mainland Britain, because of constraints on mail transfer of sperm samples. Provision of the data required approval of the study by the Privacy Advisory Committee. Multicentre Research Ethical Approval was also required to allow us to approach the relevant Health Boards for current GP address, and request patient contact.

Male subject questionnaire

In the initial mailshot to prospective participants it was emphasised that varying degrees of participation are possible, and that this is entirely dependent on the participant's continuing consent. It has also been emphasised that even minimal participation is of great value. Those indicating willingness to participate were sent a questionnaire, addressing birth order and age gap between themselves and next oldest sibling, fertility, health, occupation, duration of residence at current address, exposures to chemicals, lifestyle and stress. At the end of the questionnaire, respondents are asked if they would be prepared to participate in the reproductive health assessment by providing a sperm sample, in which case the sample pack and instructions are sent by mail. In addition, the participant is asked whether his mother is alive and could provide details of the time when she was pregnant with the participant by answering a brief questionnaire forwarded by the participant.

Maternal questionnaire

Respondents agreeing to forward a questionnaire to their mothers would be sent the mother's questionnaire and necessary freepost envelopes for return. The covering letter will make clear

that we did not have her address, nor need it, and that whether or not she completes and returns the questionnaire is entirely her own decision; her son would not know.

Semen analysis

A major problem in constructing a near-random survey of semen quality has been the need for subjects to deliver a semen sample to a clinical facility close to an appropriate laboratory. This has hitherto limited the geographical scope of data on semen quality, and is a substantial barrier to subject recruitment. We have overcome this problem by the use of a novel and unique transport medium developed and validated by our group, which allows semen samples to be produced at home, and posted to a central analysis laboratory, without significant loss of quality. Subjects who have agreed to participate in the study are mailed a semen collection kit based on this medium, together with detailed instructions on sample collection, abstinence, and the return of the sample to the central analysis laboratory. Samples are analysed according to the WHO criteria for ejaculate volume, sperm concentration, sperm motility and morphology, together with computer-assisted assessment of sperm motility.

2.2.2 Meeting presentation and discussions

The project

To complement their questionnaire data men participating in the study are also given questionnaires to be passed on to their mothers. As the survey is not aimed at men with an identified fertility problem, it is hoped there should be little difficulty in asking the men to involve their mothers in this way.

The study concentrates on young men because if there is an ongoing secular decline in semen quality it will be most evident in the youngest men. Furthermore, there is an increased likelihood of being able to obtain information from participants' mothers, and study findings (semen quality and exposures) will be most relevant to the upcoming generations of men (today's male infants and children).

In addition to the main surveys there will be a matched pairs sub-study, looking at twin pairs, pairs of non-twin brothers, and matched pairs of non-brothers. In the twin study, no attempt has been made to distinguish monozygotic and dizygotic twins. The major reason for the twin

group is to investigate pairs of men who had an identical intra-uterine environment, which twins share irrespective of whether or not they are identical. Little information is available on the genetic component of fertility.

Sampling and recruitment

The process of subject recruitment is complex. Gaining access to such a large random sample has proved to be much more difficult and more time consuming than expected. Approvals have been required from the Multi-Regional Ethics Committee (MREC), the relevant Local Ethical Committees, the Privacy Advisory Committee of the General Register Office (GRO) for Scotland, the Data Protection Registrar and the Director of Public Health (DPH) of each of the Scottish Health Boards.

One of the conditions of being provided with a random sample of male births was that the GRO would not give the study team direct access to subjects' names and addresses, that is the study team only obtains a name and address when a man agrees to participate in the study and provides contact details directly to the research team. This has meant that a member of the study staff has had to be seconded to the GRO to collate and mail out, to each GP, invitation packs (addressed by sticky labels provided by GRO) for all men on that GP's list who had been selected into the random birth sample (n = approx. 10 000), or who were one of all the twin births that could be identified (n = approx. 3000). GPs have been asked to forward the post-paid invitation packs to the men, unless there is a serious contraindication, in which case the pack(s) are to be returned to the GRO (to preserve confidentiality of addresses). In order to be able to track recruitment attrition GPs have also been asked to complete and return an action sheet, indicating the numbers of packs forwarded and returned.

The invitation packs posted on by GPs give brief information about the study and request the man to provide a name and address to the research team, on the enclosed reply slip, so that he can be mailed a questionnaire and more detailed information about participation in the study. The reply slip also requests very limited demographic information (employment status, number of brothers, number of offspring if any). All men are asked to return the reply slip to the research team, but those who do not wish to participate in the study do not provide a name and address, so their replies are anonymous. However, a serial number on the reply slip

will allow limited demographic information (postcodes only) to be ascertained for men who decline to participate in the study, thus enabling some comparison between the participant and non-participant groups.

Developing study materials

As there is no face to face contact with the subjects, especial care has had to be taken to produce materials and information that motivate the men contacted to take part in the study. The initial contact material, for example, makes no mention that a semen sample may be requested, though it does explain the background to the study and some possible explanations for effects that have been observed. Focus groups were used to help develop the materials, with participants recruited through a range of employers (NHS, armed forces, private industry). A problem was identified with the covering note from the GRO that (as a further condition for the study) had to be sent to GPs with the researchers' letter eliciting their help, and also had to be included in the men's invitation packs. By reporting the focus group's views on the note to the GRO, and making limited constructive suggestions of possible solutions, a suitably 'user-friendly' letter was finally developed. There is also a Web site[a] that men taking part can consult, which it is hoped will maintain participant 'loyalty'.

Response rate and recruitment

It had initially been hoped that 10% of those mailed invitations would in the end agree to provide semen samples; to achieve this it was estimated that a substantially higher proportion, around 40%, would need to reply with addresses, and about three quarters of these (30% of total) would need to return completed questionnaires. It is too early to determine the response rate, as the invitations are still in the process of being mailed out, and it is not known how long it takes for a GP to decide whether to forward information to the men identified. Currently about 25 responses are received per day, but if this rate persists, despite accumulating invitations dispatched, the response rate may prove to be below the initial target. Only about one quarter of those who have replied have declined to participate in the study (compared with the 60% non-participation anticipated), but it is likely many non-

[a] URL (May 2000) http://www.med.ed.ac.uk/hew/repro/

participants will not bother to return the ‘blank’ reply-slip, despite the request to do so. The response rate will be reviewed once all invitations have been sent and the pattern of responses across time can be plotted.

Questionnaires

The questionnaire covers several issues; for example, questions seek to identify a range of potential exposures through various routes in early and later life, relevant past medical history and current state of health and fertility history, as well as lifestyle factors, including diet and current occupation. Questions on occupation focus especially on occupations which might be potentially harmful to reproductive health, but are balanced by 'dummy' questions. A first batch of 20 questionnaires has very recently been sent out to men agreeing to participate in the study, and of these three have been very promptly completed and returned. These three men have all indicated that they would be prepared to participate further in the study, that is they can now be contacted with a request for the provision of a semen sample. It remains to be seen what the overall agreement rate will be.

Collection of semen samples

The study has been made possible by the development of new technologies enabling transportation by post of semen samples collected at home. However, severe difficulties have been encountered as a result of changes in Royal Mail regulations for packaging of biological fluids, such as semen samples. Regulations now require unwieldy and expensive plastic drums and boxes, and this has necessitated changes to the logistics of sample collection and the rewriting of sample instructions. Despite meetings and discussions with Royal Mail ‘specialists’ since mid-1998, the changes were not notified to the study team, but discovered by chance.

The MREC advised non-disclosure to participants of the results of their semen analysis. This should help to rule out bias that could result from self-selection by men concerned about their fertility and desirous of a semen analysis.

Database development

Databases are being developed to allow monitoring of responses to all mailings, and to assist in the complex logistics of timing of mailings.

The next stage

Given the ground-breaking nature of this research, no published evidence about likely completion rates at the various stages was available to inform research design. In the next few months there will be a rigorous assessment of the response rate to mailed invitations, the rate of consent to the provision of a semen sample and the success in obtaining such samples. If the resulting figures are ascertained as being too low to make it likely that the numbers aimed for will be achieved, then decisions will need to be made regarding strategies to compensate for the shortfall.

2.3 TRENDS IN HYPOSPADIAS PREVALENCE IN UK AND EUROPE: AN ASSESSMENT AND ANALYSIS OF EXISTING SURVEILLANCE DATA

Principal investigators - Dr Martine Vrijheid,[1] Dr Helen Dolk,[1] Dr Ben Armstrong,[1] Ms Beverley Botting,[2] Dr David Stone,[3] Prof Elisa Calzolari,[4] Mr John ES Scott, [5] Dr Martie van Tongeren[6]

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[4] President EUROCAT Association, Emilia Romagna EUROCAT Register, Italy
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[6] Institute of Occupational Health, University of Birmingham.

2.3.1 Abstract

Introduction

Hypospadias is a congenital abnormality of the male genitalia characterised by incomplete development of the urethra, so that the external meatus is located at a variable point on the ventral surface of the penis proximal to the tip of the glans. In the most proximal positions, the meatus may be in the perineum: in the most distal there may be only an incomplete prepuce. Proximal displacement of the urethral meatus is usually accompanied by a variable degree of ventral flexion of the penile shaft, known as chordee.

There is some evidence that the prevalence of hypospadias has been increasing, and that there has been a concomitant increase in related abnormalities such as cryptorchidism and testicular cancer, as well as a fall in male fertility. A hypothesis has been proposed that the underlying cause of the change in all these conditions may be exposure to EDCs, including xenoestrogens. Potential EDCs include dioxins, polychlorinated biphenyls (PCBs), and organochlorine pesticides, and also dietary phytoestrogens (such as in soy products). Whether or not this hypothesis will be supported by further research, there is a need to establish where, when and to what degree the prevalence of hypospadias has been increasing, to continue surveillance of hypospadias, and to investigate the various potential causes of any increase.

While a number of congenital anomaly registers in UK and Europe register hypospadias, it is known to be difficult to interpret the resulting data since practice concerning the notification and registration of distal (sometimes called 'minor') forms of hypospadias is very variable. Distal forms probably represent around three quarters of all cases, so that lack of standardisation in their registration has a potentially great effect on recorded prevalence. Existing surveillance data need to be analysed with this problem in mind, and registration policy and practice need to be examined to establish the basis for a valid account of hypospadias trends in the future.

Study objectives

The study has the following objectives:

- To assess the quality of Office for National Statistics (ONS) and European Congenital Anomaly Register (EUROCAT) data on hypospadias, with regard to completeness of ascertainment, validity and standardisation;
- To identify temporal trends, and seasonal, geographical, and socioeconomic differences in prevalence of hypospadias in ONS and EUROCAT data;
- To analyse the relationship between prevalence of hypospadias and occupation of the mother, as recorded in ONS and EUROCAT data, particularly with regard to exposure to EDCs; and
- To publish an interpreted set of ONS and EUROCAT data on hypospadias in the scientific literature; and
- To establish a basis for future collaborative European studies of hypospadias epidemiology.

Study design

Data from two large surveillance systems, the EUROCAT network and the ONS congenital surveillance scheme, will be assessed and analysed from 1980–1996. Together these systems survey around 1 million births per year. Analyses will concern classification by severity and data collection characteristics and differences in prevalence by year, place, season, and socioeconomic group. In addition, data on occupation of the mother recorded by the two

systems will be analysed in relation to the prior hypothesis that exposure to EDCs carries an excess risk for hypospadias.

Progress

Data validation

Data validation activities aim to determine how complete the ascertainment of hypospadias is in ONS and EUROCAT data, as well as how ascertainment varies by type (distal or proximal) of hypospadias. Analyses of time trends and geographical differences in ONS and EUROCAT hypospadias data will highly depend on the results of data validation. The aim is to make the limits of interpretation clear in published data, on an individual registry by registry basis, thus documenting the often unwritten knowledge about changes in ascertainment or case definition.

EUROCAT data validation

A questionnaire on hypospadias registration policies and practices has been designed and sent to 35 congenital malformation registries; 27 have now returned the questionnaire. EUROCAT officially excludes glandular hypospadias, which is also the most common form (approx. 75% of cases). The results of the questionnaire show a wide range in factors such as when and by whom hypospadias is notified, how decisions about in/exclusion are taken, and what is done in practice with cases for which the type of hypospadias is not specified on the notification. These differences are reflected in the wide range of prevalence rates for hypospadias found in EUROCAT registrations. The smallest prevalence rate (0.36/1000 births) is found in Galway (Ireland), the largest (2.57/1000 births) in Mainz (Germany).

A sample of over 350 cases born between 1995 and 1996 will be selected in 12 EUROCAT registries that are able to participate in further case validation. A case description questionnaire has been developed to collect information on the exact diagnosis of the hypospadias (position of the meatus, the degree of flexion in the penile shaft) and on the age at surgery. This case questionnaire will be sent to the paediatric surgeons, paediatric urologists or plastic surgeons who treated these cases.

ONS data validation

Previous validation studies of ONS data have shown severe under-ascertainment of congenital anomalies by ONS, and it is known that district health authorities vary in their notification rates depending on the system of case ascertainment they have set up. For validation of ONS data a small number of districts will be selected and cases born in recent years in these districts who have undergone paediatric surgery for hypospadias will be identified (by consultation of paediatric surgery records). The proportion of these children who were notified to ONS will be determined. Case description questionnaires will be completed for these cases by paediatric surgeons. The collection of cases operated at a number of paediatric surgery units is underway. The centres involved are Newcastle, Southampton, Sheffield and Birmingham. In the selected districts, cases notified to ONS but not identified from paediatric surgery records will also be followed up to determine their case status and severity.

Analysis of occupation of the mother

Methods

ONS data were analysed by occupation of the mother, by calculating the prevalence of hypospadias according to individual occupation codes, and by an exposure likelihood classification, created from a job exposure matrix for EDCs that has been developed for this study. In the job exposure matrix, three occupational hygienists classified jobs into three categories of unlikely, possible and probable exposure to seven groups of EDCs.

The following exposure categories were used:

- Very unlikely that exposure occurred amongst workers with this job title;
- Possibility that some of the workers with this job title had exposure (but the probability is fairly low); and
- Probability exists that at least a proportion of the workers with this job title had some exposure.

Cases for the occupational analyses are all cases of hypospadias (ICD9 7526)* registered since 1980 for whom occupation of the mother was coded.

We use two types of denominators:

- All congenital anomalies (ICD9 740-759) registered on the ONS congenital anomaly database, analysing the proportion of hypospadias cases out of the total of all congenital anomalies — the use of all congenital anomaly cases as the denominator limits bias through differences in coding of the mother's occupation between congenital anomaly data (from hospital maternity notes) and birth (from birth registration);
- 10% of all births in England and Wales for which the occupation of the mother is coded.

In 1990, ONS accepted stricter criteria for 'minor' congenital anomalies, which affected registrations of both hypospadias and all congenital anomalies. A sharp decrease in hypospadias rates and rates of all congenital anomalies can be seen in 1990 and 1991, with rates stabilising thereafter. Analyses of hypospadias cases as a proportion of all cases of congenital anomaly were therefore carried out for the periods of 1980–1989 and 1992–1996 separately and these two periods combined, (excluding 1990 and 1991 when the proportion was highly affected by the introduction of the new exclusion criteria). Analyses using 10% of births for which maternal occupation is coded will be based on the 1992–1996 period only, when the quality of coding of maternal occupation in the births data was relatively good.

The coding of occupation of the mother in the ONS congenital anomaly data is known to vary greatly by district health authority reporting the cases. Districts with poor occupational coding (less than 70% of congenital anomaly cases) were excluded from occupational analyses. Overall, the percentage of congenital anomaly cases for which maternal occupation was not coded was 69% in districts with poor occupational coding and 12% in the other districts. Exclusion of districts with poor occupational coding resulted in exclusion of 36% of all cases of congenital anomaly (52803/146445) and 18% (7093/39585) of cases with occupation of the mother coded.

* ICD code taken from Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death (Ninth Revision), Geneva, World Health Organization, 1977

Logistic regression was used to analyse the proportion of hypospadias cases out of the total of all congenital anomaly cases by occupational codes (individual job titles) and by exposure categories. Adjustments were made for potential confounders — year of birth, region, maternal age, social class of mother and social class of father. Analyses were done both with and without adjustment for social class because of the possible danger of over-adjustment, as social class variables are constructed directly from the occupational codes. Analyses using births as denominator will follow similar methodology.

Preliminary results

In the 1980–1989 period there were 2797 cases of hypospadias out of a total of 26 456 cases of congenital anomaly (10.6%). In the 1992–1996 period there were 679 hypospadias cases out of total of 6035 (11.3%). Analyses of the proportion of hypospadias cases by 277 individual occupational codes showed that several O/E ratios were nominally statistically significant, but a global test of heterogeneity showed no more variation in ratios overall than could be explained by chance ($p>0.20$). Occupations with nominally statistically significantly raised O/E ratios were management consultants (1980–1989, and 1980–1989 and 1992–1996 combined), physical and geological scientists (1980–1989), tailors, dressmakers (1980–1989, and 1980–1989 and 1992–1996 combined), hairdressers (1992–1996), vocational and industrial trainers (1980–1989 and 1992–1996 combined) and credit controllers (1980–1989 and 1992–1996 combined). Raised O/E ratios in individual occupational groups must be interpreted with extreme caution due to the large number of comparisons made, and the lack of evidence for heterogeneity overall.

Odds ratios by categories of maternal occupational exposure to EDCs (1980–1989 and 1992–1996 combined) show no evidence for an increased proportion of hypospadias cases in the 'possible' or 'probable' exposure category compared with the 'unlikely' exposure category for any of the seven substance groups separately, or for exposure to the seven substance groups combined (exposure to *any* substance). There was no evidence for an upward trend with the likelihood for exposure in any of the substance groups. Adjustment for confounding factors did not substantially change results. Results for the 1980–1989 and 1992–1996 periods separately also showed largely negative results. An exception to this is the 1992–1996 period where we find a statistically significant trend with likelihood of exposure to phthalates before

social class adjustment (OR exposure group 'probable' versus 'unlikely' 1.53, 95% CI 1.05–2.21; p for trend = 0.02) but not after adjustment (OR exposure group 'probable' versus 'unlikely' 1.25, 95% CI 0.80–1.95; p = 0.26).

Further analyses will focus on the use of the 10% of births for which occupation is coded as denominators in the analyses of occupation and exposure categories. In addition, the analyses by occupation of the mother will be repeated for EUROCAT data in centres where coding of maternal occupation was reasonably complete.

2.3.2 Meeting presentation and discussions

Problems identified

There is considerable concern that analyses of data on hypospadias may be adversely affected by misclassification or inconsistencies in the reporting of congenital anomalies at the time of birth. Ideally, clear and detailed criteria for classification of anomalies should be applied consistently between districts and for an extended time period. Unfortunately, the available data are less than ideal. The extent of the problem should become apparent when the ongoing validation studies are completed.

The method used to estimate maternal exposure to specific chemicals is necessarily crude, being based on the recorded occupation code for the mother. These codes are themselves rather broad categories and, within a given occupation, potential exposure to chemicals could vary considerably. In addition, no account can be taken of the timing or duration of possible exposure in relation to pregnancy, a factor which may be very important in the induction of hypospadias. Another confounding factor is variation in possible environmental exposure to EDCs, for example through the diet (contaminants or phytoestrogens).

2.4 ENVIRONMENTAL RISK FACTORS FOR HYPOSPADIAS: A POPULATION-BASED CASE–CONTROL STUDY IN THREE HEALTH REGIONS

Principal investigators - Professor Paul Elliott,[1] Dr Mark Nieuwenhuijsen[2]

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2.4.1 Abstract

Introduction

Here we describe a case–control study of hypospadias in three English health regions. Specific questions to be explored include maternal and paternal (before conception) exposure to occupational and household chemicals and maternal exposure during pregnancy, use of oral contraceptive pill including whether pill use continued into pregnancy, parental use of recreational drugs and/or prescribed medication, time to pregnancy as an indicator of relative fertility of the couple, water consumption and potential exposure to recycled water through the domestic supply (based on water zone of domicile), and urban/rurality, seasonality and distance from agricultural land, as potential markers of exposure to agricultural chemicals. Other potential explanatory variables/confounders include family history of hypospadias and other malformations, dietary intake (especially food of plant origin and vegetarianism), smoking, use of alcohol (particularly during the early pregnancy), parity, birth order, maternal infection during pregnancy, maternal and paternal age, and ethnic origin. Cases will be identified using multiple sources of ascertainment through a project already funded by the Wellcome Trust on the geographical epidemiology of hypospadias, giving a reliable estimate of birth prevalence. Population-based controls for the present study will be selected at random from ONS birth register data. Around 300 cases and 300 controls are to be included. With 15% exposure prevalence among the controls, the study has 80% power at the 5% level of significance to detect a relative risk of around two for exposed versus unexposed individuals, for example, whether or not there has been household or occupational exposure to pesticides.

Work which has led up to the project

There are concerns about trends over the last 50 years in some indicators of male reproductive health. The evidence is strongest for a rise in the incidence of testicular cancer[1] but there is also evidence that sperm counts may have declined in parts of Western Europe.[2] Other reproductive related disorders also appear to have increased over the same period. Sharpe has postulated the involvement of oestrogens in disorders of the male reproductive tract, including hypospadias.[3]

Previous reports have shown geographical and seasonal variation in the birth prevalence of hypospadias,[4] suggesting the importance of environmental factors in its aetiology. However, it is uncertain to what extent this reflects true differences in prevalence or variation in levels of ascertainment. There also appears to be a genetic component, with aggregation of cases within families and occurrence as part of complex genetic syndromes.[4] An animal model of hypospadias (Boston Terrier) has been reported. There is weak evidence from population-based observational studies implicating the role of maternal factors in the aetiology of hypospadias. In one large study, high maternal age and low parity were associated with risk ratios up to 1.5. It should be emphasised, however, that these data were derived from national registries that have been poorly validated and are known to be incomplete.[4] Other factors that have been investigated include those related to pregnancy and birth, for example low birth weight, gestational age at birth, caesarean delivery and primiparity.[4]

Toxicological evidence suggests that genital abnormalities such as hypospadias can result from exposure to various environmental pollutants. For example, hormonally active drugs like the synthetic oestrogen, diethylstilbestrol (DES), Danazol (androgenic) and progestins cause urogenital malformations in the reproductive tracts of humans (e.g. DES) and rodents. Pesticide residues have also been implicated as potential hormonal disrupters,[5] and anti-androgenic drugs and fungicides have been found to induce alterations in phenotypic sex differentiation. Effects such as hypospadias, ectopic testes, vaginal pouches, agenesis of the ventral prostate, and nipple retention in male rats have commonly been observed in the laboratory.[6] However, epidemiological evidence is weak and no firm link between environmental oestrogens, or xenobiotics with a hormonal action, and human reproductive health effects (including hypospadias) has been established.

Here we are in the process of carrying out a case–control study of hypospadias in three English health regions (Eastern Health Authority Regional Office, London Regional Office and half of South East Regional Office: South East will be West Surrey, West Sussex, East Surrey, East Sussex, Brighton and Hove, West Kent and East Kent). It will build on work already funded by the Wellcome Trust to establish a high quality case register of hypospadias based on multiple sources of ascertainment and to describe the geographical (small area) epidemiology of this condition. Specifically, a grant from the Wellcome Trust supports a Research Fellowship in Clinical Epidemiology for Dr Paul Nelson, to establish the case register and to carry out the geographical analysis.

The current study takes advantage of this population-based prospective case register to move from the ecological (small area) level to the individual level, using a case–control design.

Study objectives

The aims of this study are to explore the 'endocrine disrupter' hypothesis in the aetiology of hypospadias. Specific areas are described below.

- Both maternal and paternal exposure to occupational and household chemicals, before the pregnancy and during the pregnancy (mothers only), by use of a questionnaire at telephone interview. Chemicals will be grouped using chemical inventories into major classes, for example, pesticides, solvents, cleaning agents and disinfectants.
- Use of oral contraceptive pill. Questions will include type, duration of use and whether pill use continued into pregnancy.
- Maternal or paternal use of recreational drugs and/or prescribed medication prior to the pregnancy, and during pregnancy (mothers only).
- Time to pregnancy as an indicator of relative fertility of the couple.[7]
- Water consumption, type (boiled drinks, tap or bottled), quantity, and potential exposure to recycled water through the domestic supply (based on water zone of domicile and occupation).
- Urban/rurality, seasonality and distance from agricultural land, which may be potential markers of exposure, for example, to agricultural chemicals.

- Other potential explanatory variables/confounders, such as family history of hypospadias or other congenital anomalies, dietary intake (especially food of plant origin and vegetarianism), smoking, use of alcohol (particularly during the early pregnancy), and residential histories (including address at time of conception), parity, birth order, maternal infection during pregnancy, maternal and paternal age, gestational age at birth, type of delivery, birth weight and ethnic origin.

Study design

The study builds on a high quality case register, already funded by the Wellcome Trust, as part of a study of the geographical (small area) epidemiology of hypospadias. Through multiple sources of ascertainment, the Wellcome-funded study will overcome many of the problems of under-ascertainment that have affected previous studies based on routine registries and will give a reliable estimate of the birth prevalence of hypospadias.

Study population

All births within the Eastern Health Authority Regional Office, a London Regional Office and half of South East Regional Office (South East will be: West Surrey, West Sussex, East Surrey, East Sussex, Brighton and Hove, West Kent and East Kent), occurring in an 18 month period will be included.

Selection of cases

Case definition

This has been developed in association with Mr Pierre Mouriquand, consultant paediatric urologist at Great Ormond Street Hospital, who is collaborating in the study. Three main categories can be identified.

- Glandular — the meatus is distal to the corona and there is usually no chordee.
- Anterior hypospadias without chordee — the meatus is at any position between the corona of the glans and the mid-shaft penis, the chordee, when it exists, is distal.
- All other hypospadias with a marked chordee — in these cases, the position of the meatus can only be defined at the time of surgery, after correction of the chordee.

These categories will be further classified by association with other abnormalities, and by family history of reported urogenital abnormality.

Case ascertainment

The Wellcome Trust funded study includes identification of patients from the following sources.

- Notification (validated against case notes) by all surgeons who operate on hypospadias cases in the regions, theatre records, and visits to relevant hospitals, including in neighbouring regions to deal with cross-boundary flows.
- Access to the National Congenital Anomaly Register (NCAR) which is maintained by the ONS and is held in this department in the UK Small Area Health Statistics Unit (SAHSU), as well as access to local malformation registries.
- Hospital admissions (HES) data, also available in SAHSU.

We intend to interview mothers of affected infants (and those of controls) as part of the case–control study (the Wellcome study does not include direct contact with the parents of cases).

Selection of controls

We are using population-based ascertainment of one healthy control per case through GP surgeries that lie within the study regions and are picked at random from the ONS birth register. Controls will be eligible for the study if they are male and their parents lived in the study region at the time of conception. Population-based healthy controls have the advantage of avoiding possible selection biases associated with hospital controls, since different general practitioners have different referral practices to hospitals. In addition, many of the hypospadias cases will be found in tertiary referral centres such as Great Ormond Street, where the case mix of other (control) conditions will be atypical.

Field methods

A modification of published questionnaires concerning exposure to occupational and household chemicals will be developed. A lecturer in environmental epidemiology at Imperial College, Dr Mark Nieuwenhuijsen, is the co-principal investigator. He has considerable experience in occupational studies of chemical exposure, including the development of

exposure questionnaires for use in case–control studies, job type coding and estimation of exposure levels, including pesticides. He, Dr Tina Jensen and Dr Paul Nelson have developed an exposure questionnaire (with reference to successful published questionnaires[8,9]) to obtain information on exposure to occupational, environmental and household chemicals. He will also conduct the job coding and oversee the use of a chemical inventory together with Dr Nelson.

A nutritional questionnaire based on published work will also be used to obtain information on potential dietary confounders including food of plant origin and vegetarianism.[10] Nutritional expertise is available in the department. A detailed water and alcohol consumption history will be obtained.

A questionnaire has been designed by Dr Nelson using Microsoft Access, and data will be entered directly into a lap-top during the telephone interview by the research assistants. Data entry will be subject to strict quality control and internal consistency checks, including use of relational database techniques. Data will be downloaded daily to a secure desk-top computer. This will avoid the need for separate data entry and reduce the risk of error during data input.

The water industry has agreed to provide data on water quality at the level of the water supply zone for the geographical study. A measure of water reuse, as a possible marker of contaminants by oestrogenic compounds in the water supply, is being developed in a collaboration between this department and the water industry. It will be used as a potential explanatory variable in this study, by assigning the water supply variables at the level of the water supply zone to individuals within that zone. Information on individual household water consumption will be obtained by questionnaire (type of drink and quantity of consumption).

Permission and confidentiality and consent

The department has permission to use the ONS data set for SAHSU and related projects. Specific agreement for this study is being sought for the congenital malformations data. The department also has permission to use HES data for England. Permission to obtain data from the participating hospitals and GPs in the three regions is being sought from the central St Mary's Ethics Committee and will be sought from each of the relevant ethics committees.

Data will be downloaded to a central computer each night. Names will be removed and identification will be by number only. No names of cases or controls will appear in published reports. Consent to approach cases will be sought from the surgeons and GPs involved in their care. Consent to approach controls will be sought from their GPs. Individual parents will then be asked for consent before the telephone interview takes place.

Data analysis and statistical methods

An analysis for unmatched case–control studies will be carried out,[11] stratifying for potential confounders as appropriate. Multiple logistic regression analysis will also be performed[11] using the software package Stata.

Sample size and power calculation

Assuming a rate of occurrence of 3 per 1000 live births, based on the ONS 1991 census, HES and NCAR data, the study will ascertain around 260 cases over an 18 month period. Assuming a 70–80% response rate, we will need to recruit around 200 controls with one control per case. With 80% power and 5% level of significance, for an exposure affecting 15% of controls, this sample size would be sufficient to demonstrate an odds ratio of 2 for the exposure of interest, for example, whether or not there has been household or occupational exposure to pesticides.

Project management and steering committee

Day to day management of the study will be carried out by Dr Paul Nelson who will oversee the recruitment of the cases and controls. With Dr Nieuwenhuijsen, he will be responsible for training the two research assistants in all aspects of the study procedures and in the interviewing techniques. Dr Nelson will also oversee and manage the daily activities of the two research assistants. A project team comprising the two principal investigators, Dr Nelson and the two research assistants will meet frequently (about every 2–3 weeks) to discuss progress and deal with any problems that may arise. A steering committee will meet twice yearly to review progress. This committee will comprise Professor Paul Elliott, Dr Mark Nieuwenhuijsen, Dr Paul Nelson (Wellcome Research Fellow in Clinical Epidemiology), Dr Mike Joffe (Reader in Epidemiology and Public Health, Department of Epidemiology and Public Health, ICSM), Mr Pierre Mouriquand (Consultant Paediatric Urologist at Great

Ormond Street Hospital), Professor Euan Hughes (Professor of Paediatric Endocrinology at University of Cambridge), and Dr Jon Wakefield (Senior Lecturer in Statistics at Department of Epidemiology and Public Health, ICSM).

Project Timetable

The timetable for the project is outlined below.

- 01.10.97 — Start of Wellcome funded case ascertainment study
- 01.10.98 — Start of case–control study
- 01.01.98–31.03.99 — Development of questionnaires and data entry programs
- Recruitment and training of research assistants
- 01.01.97–30.09.98 — Eligibility of cases and controls. Boys born during this eighteen month period whose mothers lived in the study region at the time of the birth are eligible. Inclusion of cases who were born from 01.01.97 is made possible since most ascertainment (through referral to surgeons) occurs beyond 9 months of age. For the few cases who will be referred before 9 months, a retrospective examination of operation notes and booked operations will be undertaken.
- 01.10.97–31.03.99 — Recruitment of hospitals and surgeons, setting up of systems and database, and ascertainment of cases
- 01.02.99–31.03.2000 — Controls sought from GPs and health visitors serving the study areas by a weighted random selection of GPs. The weighting will be based on the size of the practice's paediatric lists. From 31.03.98 the research assistants telephone parents of the cases and controls.
- 01.04.2000–31.08.2001 — Late ascertainment of cases and controls, and calls to parents (Dr Nelson), statistical analysis and writing up.

Quality assurance

Training of research assistants in all research methods and interview techniques will be carried out. Direct data entry will be employed with built in rigorous quality assurance including internal consistency checks and the use of relational database based questionnaire. The

Steering Committee, incorporating external members, has been set up to review the progress and monitor results. A project team comprising the principal investigators, Dr Nelson and the two research assistants will meet approximately every 2–3 weeks throughout the study. A panel of experts chaired by Mr D Wilcox is being set up to ensure appropriate diagnosis and classification of cases.

Added value of the research

This study builds on an existing study which already supports the salary of Dr Nelson. It also covers the cost of case ascertainment, review of hospital notes, a computer, and database and analysis software. The expertise of Professor Elliott and Dr Mark Nieuwenhuijsen are also being made available without cost to the project.

2.4.2 Meeting presentation and discussions

Study population

This project uses a case–control design, with one healthy control per case, drawn from the general population. The study builds on a case register, already funded by the Wellcome Trust, that draws on multiple sources of ascertainment, including notifications by surgeons and hospital admissions data. To date, 160 cases have been identified in south-east England. Controls are to be selected at random from ONS records; hospital-based selection was not considered appropriate because of possible linkage with other medical conditions.

Recruitment to the study is in its final stages of organisation and interviews are to commence imminently (as of October 1999).

Bias could be introduced in the responses, either by the interviewer knowing that the subject is a case or a control or by the parents of hypospadias cases realising the purpose behind the questionnaire. Every effort will be made to ensure that neither the interviewer nor the subject is aware of the case or control status of the latter. However, should the subject realise they are a case, the interviewer will reassure them the study is hypotheses generating only.

The questionnaire

An exposure questionnaire has been designed to obtain information on exposure to occupational, environmental and household chemicals, and on diet, water and alcohol intakes.

Data are entered directly into a Microsoft Access database, using a lap-top computer and later transferred to a desk-top computer.

Obtaining ethical approval for the use of the questionnaire has proved difficult. Some issues, for example the use of recreational drugs or previous miscarriage, are particularly sensitive. The MREC adopts an iterative approach to approval. Following MREC approval the study was submitted to more than 50 local ethical committees (LREC). Although about 40 LRECs had no difficulties with the proposed study, ten had specific problems that could only be resolved by changing the study protocol. Objections were raised about the intrusiveness of the questionnaire and the possibility of engendering a 'sense of blame' among mothers of affected children (although the person administering the questionnaire would be well informed and would explain the study was hypotheses generating only). Proposals to reword some comments about the quality of the questionnaire response and to follow up by telephone those who failed to respond to the initial contact also gave rise to some objections.

Selection of controls

The issue of whether control selection should be from hospitals (e.g. fracture clinics) or on a community basis (e.g. ONS records) raises a number of issues. There is a need to assess whether the problems in gaining permission to approach individuals in the community outweigh the risk of controls from hospitals being potentially unrepresentative (e.g. likely to be in poorer health then the general population). The difficulties in choosing between the use of hospital or community-based controls are well recognised. Community controls are more representative of the general population, and a particular reason for using community controls in this survey is to facilitate area comparisons. However, using community controls can be a more expensive option and may not yield as good a response rate or such scientifically reliable responses as hospital controls. Nonetheless, there has, so far, been a good response rate from community controls in this particular study. Overall, the current trend in such studies is to use community controls.

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2.5 GEOGRAPHICAL EPIDEMIOLOGY OF PROSTATE AND TESTICULAR CANCER IN GREAT BRITAIN

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2.5.1 Abstract

Introduction

Cancer of the testis is a malignant tumour, which mainly affects young men, with a peak incidence at around 30 years of age. A second increase in incidence occurs after the age of 50. It is the most common cancer among young men.[1] The incidence rates have increased during recent years in many countries.[2,3]

There is a geographical variation in the incidence rates worldwide. The highest incidence rates have been noted in Denmark, Norway and Switzerland, whereas the lowest rates are seen in eastern Europe and Asia.[4] An almost tenfold geographic variation within the Baltic Sea countries has been observed.[2]

There is concern that the increase in testicular cancer may be linked to environmental exposure to chemicals, including EDCs.[5] Exposure of pregnant mice to ethynyl estradiol increases the frequency of cryptorchidism and testicular cancer.[6] Exposure of humans and animals *in utero* to DES resulted in an increased incidence of cryptorchidism, a recognised risk factor for testicular cancer.[7,8] Occupational exposure to chemicals in the petroleum and natural gas industry, printing industry, leather industry and military aviation repair have also been implicated, as well as occupations with exposure to polycyclic aromatic hydrocarbons, metals, metal dusts and, possibly, cutting oils.[9]

Prostatic cancer incidence rates, but not mortality, have also increased during the last decades.[10,11] At least in part, this apparent increase may reflect improved detection rates.[12,13]

The aetiology of prostatic cancer is not well understood, but associations with endocrine factors, diet, nutrition, sexual and reproductive factors, as well as environmental exposures,

have been reported.[14–17] Studies have linked prostate cancer to occupational exposures to radiation and radionuclides.[18,19] High risks have also been reported for workers in the cadmium, farming and rubber industries.[14,15] Oestrogens are used in the treatment of prostate cancer and induce tumour regression. Environmental exposure to xenoestrogens might therefore be expected to reduce the incidence of prostate cancer. However, environmental anti-oestrogens could theoretically have the opposite effect.

Recent evidence indicates that a group of environmental chemicals mimicking oestrogens (EDCs) may affect reproductive health.[5,20–24] There is a suggestion of adverse trends in human male reproductive health, but epidemiological evidence for such effects is both controversial and sparse.[25]

Study objectives

Human exposure to chemicals in the environment is unlikely to be evenly distributed geographically because of variations in industrial activity and human behaviour. If environmental chemicals have adverse effects on male reproductive health, they may thus give rise to geographical clustering of disease. This study describes the spatial and temporal variations in testicular and prostate cancer across Great Britain.

Study design

Registrations of cancers of the prostate (ages 45–64) and testes (ages 20–49), ICD-8 and ICD-9 codes 185 and 186, respectively, for the whole of England, Wales and Scotland, were extracted from the national data set held by SAHSU. Cases without a valid postcode were excluded. Denominator populations from the 1981 census were used prior to 1981, with apportionment according to annual district level estimates from the ONS. For the more recent years (1985 onwards), the 1991 census data were used.

Temporal trends across Great Britain were examined using three-year moving averages.[26] Cancer registration data were included between 1974 and 1991 and mortality data between 1981 and 1997.

Spatial trends were examined across electoral wards from 1975 to 1991. Data for 1974 were excluded because postcodes in Scotland were not in common usage until 1975. For one ward

in England, 42 cases of testicular cancer were recorded. Further investigation showed that these cases were assigned the geographical coordinates of a local military hospital and, therefore, this ward was excluded. In total, 10 530 wards in Great Britain were included in the analysis.

The Carstairs' index[27] was used as an index of socioeconomic deprivation at the electoral ward level. This index is a combination of four socioeconomic indicators from the census — the percentage of people with no car, in overcrowded housing, with the head of household in social class IV or V, and the percentage of men unemployed. Population density was used as a measure of urbanisation. Wards were grouped into quintiles of Carstairs' and population density score.

Poisson regression, allowing for over-dispersion, was used to examine the relationship between ward-level cancer incidence and deprivation, urban–rural status and cancer registry. To investigate registry effects, the national average was chosen as the reference in the Poisson models. Statistical significance was assessed using likelihood ratio tests. Ward-specific age (categorised in five-year age bands), cancer registry and deprivation standardised expected counts were calculated using indirect standardisation.

Heterogeneity (excess variation) of disease rates was examined using the test described by Potthoff and Whittinghill.[28] This tests the hypothesis of homogeneity of risk against the alternative that the relative risks are drawn from a gamma distribution. Further analysis was based on Bayesian disease mapping techniques to stabilise risk estimates because of small numbers (chance variation) at ward level.[29,30] First, empirical Bayes methods were used to estimate ward level risks nationally.[31] Hierarchical Bayesian modelling was then used to produce estimates of spatially neutral and spatially structured variation.[32] Because the methods are highly computer intensive, this latter analysis was performed on a subset of the data only. We chose the inner part of the North West Thames registry in the London area, representing a predominantly urban and suburban population, which has been extensively investigated previously at small area scale.[33–35]

2.5.2 Meeting presentation and discussions

Temporal trends

Analysis of data for Great Britain from 1974 to 1991 demonstrated that the incidence of prostate cancer in men aged 45 to 54 was consistently very low with no evidence of a time-related trend. However, in older men (aged 55 to 64), the incidence increased by 55% during the same period. There was also a substantial increase in testicular cancer incidence in men aged 20 to 49, but the increase varied from 40 to 50%.

Geographical variation

Testicular and prostate cancer incidence showed considerable variation between geographical areas (i.e. between different cancer registries), from 20% below to 35% above the national average. There was no evidence that prostate cancer incidence was related to affluence, as judged by the Carstairs' index, but testicular cancer risk was lower in areas of lower affluence. There was some evidence that testicular cancer risk was also related to (low) population density, which may be linked to affluence (see Section 3). The residual variation has been analysed using Bayesian disease mapping techniques. A subset of data (North-West Thames Region) has been analysed in detail. The results are not suggestive of a strong geographically-determined environmental effect.

One problem that may affect the analysis of data from small geographical areas is population mobility; the effect of EDCs is postulated to occur *in utero,* but cancer is detected many years later when the subject may have moved far from the source of exposure. The ONS has estimated that population mobility is relatively small; about 10% of individuals move between areas and the distance moved is generally small. A better way of analysing the data might be to link cancer cases to their place of birth but this is, in practice, very difficult with the databases available.

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3 General discussion

This section summaries the general discussions on wider aspects of male reproductive health held after the individual project reviews. In particular, discussions focused on three specific questions identified by the participants:

- What other ongoing studies in Europe are being funded by the European Chemical Industry Council (CEFIC) that relate to the investigation of environmental impacts on male reproductive health?
- What is the impact of socioeconomic status on indicators of male reproductive health, in particular testicular cancer?
- How should ethnic variations, regional variations and population migration be dealt with in epidemiological studies on male reproductive health?

3.1 OTHER ONGOING RESEARCH IN EUROPE FUNDED BY CEFIC

Participants were aware of two other current studies in Europe. A group at the University of Rotterdam in the Netherlands is studying pregnancy outcome for effects on male reproductive health. Blood and cord samples are being taken for analysis, as may be required at a later time depending on pregnancy outcome. Offspring will be evaluated for disorders such as hypospadias and cryptorchidism, and will be subject to IQ testing when they are older. A research team at the Karolinska Institute in Sweden is conducting a cohort study (from before birth) to evaluate the effects of factors such as diet, lifestyle and ethnic origin on male reproductive health. Again, biological samples are being stored for subsequent analysis.

3.2 SOCIOECONOMIC INFLUENCES ON TESTICULAR CANCER

In the UK and elsewhere, the incidence of testicular cancer appears to be related to socioeconomic status, with higher cancer rates in affluent areas than in deprived areas.[1–5] Breast cancer incidence is also positively associated with higher socioeconomic status. In contrast, the risk of hypospadias and cryptorchidism appears to be higher in more deprived

areas, but there is little literature available on the subject. The Small Area Health Statistics Unit (SAHSU) is currently undertaking a study of the epidemiology of orchidopexy (the surgical procedure to correct undescended testes), as a proxy for cryptorchidism, that will examine the association between socioeconomic status and risk of cryptorchidism. Little is known about the impact of socioeconomic factors on the incidence of testicular torsion (abnormal twisting of the testis and spermatic cord impeding blood supply).

A possible explanation for associations between testicular cancer incidence and socioeconomic status could be that testicular cancer in offspring may be related to the age of the mother at first birth. First births tend to be later in higher social groups.[6–8] Furthermore, oestrogen levels are highest at first births and this also may have some effect. Another possible explanation might be maternal nutrition; for example, fat consumption or maternal adiposity might be expected to be higher in more affluent societies. However, the influence of such factors is little understood. To investigate any possible effect of maternal nutrition and adiposity on testicular cancer incidence, it would be helpful if information on maternal weight and height could be routinely collected during studies on pregnancies and abnormalities in the reproductive system of male offspring, and this is therefore recommended.

Although mortality from testicular cancer is decreasing, the incidence of the disease is now increasing in developed countries. It is unlikely that the increased incidence is explained by mis-diagnosis of the disease. Given the age at which the disease most commonly occurs and the consequences of treatment (i.e. loss of fertility), over-diagnosis is extremely unlikely. The vast majority of cases undergo orchidectomy, and the pathology of the disease is clear cut; the incidences of both histological types (seminomas and non-seminomas, which occur equally) are increasing similarly, and both are ultimately very malignant tumours. Moreover, prior to effective treatment, mortality was increasing as well as incidence; the rise in testicular cancer mortality in England and Wales dates back to the 1920s (i.e. the 1890s birth cohort). Recent decreases in mortality may be related to people in more affluent groups being better able to take advantage of improvements in health care.

Time trends in testicular cancer incidence and mortality suggest that although diet or weight (or both) could be contributing factors, chemical exposure is less likely to be implicated.

Chemicals suggested to be endocrine disrupting chemicals (EDCs), such as DDT, were not known in the 1900s. Nonetheless it might be informative to consider whether exposure to EDCs might be linked in some way with affluence. Increased use of personal hygiene products, for example, is linked with income; use of such products may potentially have some effect if they contain EDCs. Furthermore, maternal levels of endogenous oestrogen increase with fat intake, which (as indicated above) may be associated with affluence.

It is also possible that genetic factors may play a role in testicular cancer. Testicular cancer rates are not increasing as much in men of African descent, either in Africa or America, as in men of European descent. Also testicular cancer rates are very low in Finland but high in Denmark. However, genetic factors cannot explain the time trends in cancer rates, implying there must be some interaction with environmental factors. Information from the human genome project may be informative in this area.

3.3 POPULATION VARIABILITY

Ethnic variablity

It is known that ethnicity can affect fertility, therefore accounting for ethnic variability could be particularly important in some studies in this programme. For example, it is recognised that fertility can be suppressed by male contraception moderately well in Asian populations, but with varying results in white Caucasian populations.

It is also possible that responses to different environmental agents, including EDCs, might vary between ethnic groups. Epidemiological studies could be constructed to address such a question, although unless a specific hypothesis were to be tested, the statistical power of such a study would be limited, owing to the many possible variables. Future studies might use the opportunity offered by the presence of communities of particular ethnic groups of non-European origin in some regions of the UK to investigate ethnic differences in responses to environmental agents.

Current studies conducted by SAHSU (e.g. Section 2.5), consider ethnicity as a separate variable from socioeconomic status, and it is considered a possible confounder in studies on male fertility. However, information about ethnicity tends not to be available for earlier

studies. A question on ethnicity is included in the study by Irvine *et al.* (Section 2.2), but it is likely to be less of an issue in a study of Scottish men born 30 years ago than it might be in other studies. Although ethnicity is not specifically addressed in the study by Cherry *et al.* (Section 2.1), individuals do need to be able to speak English well to participate in the study. It is also noted that ethnicity is important in the study in Rotterdam (see above), as some of the mothers included are from former Dutch colonies.

It is recognised that if ethnicity does explain a significant amount of the variability seen in semen quality, studies comparing moderate proportions of various ethnic groups require a sample size that is large enough to maintain statistical power with regard to other factors that might also be considered.

Regional variation

Regional variation in exposure to environmental agents might also be a confounder in studies on male fertility. Some studies are investigating regional differences in disease patterns that may help to identify regional environmental differences. Care should be taken when pooling health data from different regions to ensure that possible clues to regional environmental differences are not lost. The question of spatial variation has been addressed by a study on male fertility in six regions in France by Auger and Jouannet.[9]

Population migration

The smaller the geographic area considered in a study, the more population migration will affect the results. One possible way to assess the influence of migration is to analyse the same study using small and large geographical areas and then compare the results. In the study by Cherry *et al.* (Section 2.1), for example, addresses are obtained from birth certificates, so the geographical regions studied can be as small or as large as required. However, using the place of residence at one particular time as a determinant of exposure may also create difficulties for interpretation, as the exposure of most relevance to an effect may have occurred at a different period, when the subject was resident elsewhere.

One way to minimise the influence of migration is to study areas of the country where population migration is low. However, it is not necessarily easy to identify a region with a stable population, and in fact age is a greater determinant of migration than is region. In a

region where migration is predominantly outward, migration may have less of an impact and the results may be more robust than in a region where migration is predominantly inward. However, it is also possible that outward migrants might be those most likely to be exposed to agents of concern if exposure is associated with a source of employment that has been relocated.

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Annex

Programme for First Review Meeting, 27 October 1999

10.30–10.45	Welcome (Paul Harrison, IEH)
	Introduction on behalf of funding bodies (Mike Topping, HSE)
	Chairman's opening remarks (David Coggon, MRC)

Investigators' Presentations*

10.45–11.20	UK multicentre study of occupational and environmental exposure to chemicals and male fertility (Harry Moore, University of Sheffield and Nicola Cherry, University of Manchester)
11.20–11.55	Historically prospective cohort study of the Scottish male reproductive health (Stewart Irvine, MRC, Edinburgh)
11.55–12.30	Environmental risk factors for hypospadias: a population-based case–control study in three health regions (Helen Dolk, London School of Hygiene and Tropical Medicine)
12.30–13.30	Lunch

Investigators' Presentations (continued)

13.30–14.05	An assessment and analysis of existing surveillance data on hypospadias to look for trends in relation to time geographical location and maternal occupation (Paul Nelson, Imperial College)
14.05–14.40	The geographical epidemiology of testicular cancer, prostate cancer and cryptorchidism (Mireille Toledano and Lars Jarup, SAHSU, Imperial College)
14.40–15.40	**General discussion**

* Incorporating discussions on the specific projects

IEH Web Report W1 at http://www.le.ac.uk/ieh/webpub/webpub.html posted May 2000

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