The Danish National Birth Cohort – its background, structure and aim

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Background: It is well known that the time from conception to early childhood has importance for health conditions that reach into later stages of life. Recent research supports this view, and diseases such as cardiovascular morbidity, cancer, mental illnesses, asthma, and allergy may all have component causes that act early in life. Exposures in this period, which influence fetal growth, cell divisions, and organ functioning, may have long-lasting impact on health and disease susceptibility. *Methods*: To investigate these issues the Danish National Birth Cohort (*Better health for mother and child*) was established. A large cohort of pregnant women with long-term follow-up of the offspring was the obvious choice because many of the exposures of interest cannot be reconstructed with sufficient validity back in time. The study needs to be large, and it is aimed to recruit 100,000 women early in pregnancy, and to continue follow-up for decades. The Nordic countries are better suited for this kind of research than most other countries because of their population-based registers on diseases, demography and social conditions, linkable at the individual level by means of the unique ID-number given to all citizens. Exposure information is mainly collected by computer-assisted telephone interviews with the women twice during pregnancy and when their children are six and 18 months old. Participants are also asked to fill in a self-administered food frequency questionnaire in mid-pregnancy. Furthermore, a biological bank has been set up with blood taken from the mother twice during pregnancy and blood from the umbilical cord taken shortly after birth. Data collection started in 1996 and the project covered all regions in Denmark in 1999. By August 2000, a total of 60,000 pregnant women had been recruited to the study. It is expected that a large number of gene-environmental hypotheses need to be based on case-control analyses within a cohort like this.

Key words: cohort, pregnancy, life-course, epidemiology.

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INTRODUCTION

Childbearing has been a high-risk period for the mother as well as for the unborn child in all countries, and worldwide we still see (according to the WHO) 600,000 maternal deaths and 7.6 million perinatal deaths a year. Even in the most affluent societies, no less than 30% of all pregnancies end in abortions and about half of these occur before pregnancy is detected; 3–5% of newborns have congenital malformations and close to 1% are stillborn or die within the first year of life. Research on infections, drugs, environmental exposures, and vitamins such as folic acid demonstrates that some of these events are preventable. Most of the existing birth cohorts have addressed identification of causes of reproductive failures that manifest themselves before or shortly after birth. We have partly based our antenatal care prevention on results from these studies.

Birth cohorts that address perinatal outcomes are still important since many risk factors change over time. We develop new medicines, have new occupational and environmental hazards, change dietary habits and are exposed to new ways of processing food. A growing number of pregnancies are based on assisted medical intervention, which may carry new risks that have to be identified. We also develop new tools to detect infections, environmental exposures, and the role of genetic factors. These new tools provide important research opportunities and they provide new approaches to investigate the many serious reproductive failures that still have no known aetiology.

Overwhelming documentation links the condition of the infant at birth with diseases and malfunctions that manifest themselves much later in life. Fetal growth, and perhaps also factors related to fetal growth, has been associated with cardiovascular diseases, diabetes,

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obesity, infections, reduced cognitive functions etc. (1–6). Factors associated with high or low levels of sex hormones in the intrauterine environment have been associated with a number of cancers, poor sperm counts, or infertility (7–13). Some specific fetotoxic exposures may have long-lasting effects, such as mercury, alcohol, or medicine (14–16).

Diseases like diabetes, asthma, atopic diseases, and behavioural disorders in childhood may well have a perinatal aetiology (17, 18). Most congenital anomalies still have an unknown aetiology and recent findings for specific vitamins indicate that several of these conditions may be preventable.

Antenatal care (ANC) is expensive, and a large part of resources allocated to prevention and health promotion is spent on these activities, which need to be based on updated scientific evidence. Evidence-based prevention is at least as important as evidence-based medicine, especially in this period of life because of the possible long-lasting consequences (19, 20).

For these reasons we decided to establish a national birth cohort in Denmark. The planning started in 1992 and the first grant was given to the project from the Medical Research Council in 1993. We wanted to recruit the women as early in pregnancy as possible to cover the period of organogenesis and to detect as many spontaneous abortions as possible. The study aims at including no less than 100,000 pregnant women and their offspring. We wanted sufficient statistical power to examine rare diseases such as congenital malformations or childhood cancers. The study should permit follow-up for several decades for mothers as well as their offspring.

THE INFRASTRUCTURE

In Denmark, the national health services provide an excellent setting for undertaking a national birth cohort study. Denmark has a public health system in which private hospitals treat less than 3% of all patients, except for those seeking infertility treatment. Hospitals are financed by the counties and the counties also pay for the midwife service. Most of the patients are registered with a specific general practitioner (GP), who is paid by the county on a per fee basis together with a certain amount of money per registered patient regardless of the service he or she requests. Treatment from the GP is free of charge for the population that has selected this GP system (more than 95%). Less than 5% choose not to register with a specific GP. They may seek help from any GP of their choice, but in this case the GP is only refunded the standard fee from the county and the GP is free to charge the patient directly an additional amount. All activities within public and private health care are recorded and most of the information is computerized. All discharge diagnoses made in connection with hospital visits can be linked to cohort members by means of personal identification numbers (PINs).

In 1995, Denmark had a population of 5.23 million, including 1.1 million women in the age group 15 to 44 years old. They gave birth to 70,000 children that year. Perinatal mortality was 7.5 per 1,000. Infant mortality was one per 1,000. The majority of the population is of Caucasian origin, but approximately 10% of the pregnant women are immigrants. Induced abortions are legal, free of charge and available for all up to week 12 of gestation. Women over 35 years of age and those at high risk are offered prenatal screening for congenital birth defects. More than 99% of all pregnant women participate in the ANC programme through their GP and midwife, and 75% of all pregnant women have more than nine visits to their GP and/or midwife. High-risk pregnant women are always referred to departments of obstetrics at one of 43 hospitals.

AIMS

We wanted to study pregnancy complications and diseases in offspring as a function of factors operating in early life, fetal growth, and its determinants. We aimed especially at studying side effects of medications and infections. We focused not only on diseases in the period from conception to early childhood, but also on all diseases with a possible origin in the fetal time period.

The study includes data on specific exposures of interest and a biological bank. We wanted to set up a registration system of all types of medicine used during pregnancy to study perinatal short-term side effects of these drugs. We wanted to establish an exposure register that could be linked with existing disease registers. We wanted to add an important information bank to provide new research opportunities that would be available for generations of epidemiologists.

POWER CALCULATIONS

The study needs to be large to make a difference. With present incidence rates we expect the power given in Table I under ideal circumstances. Unavoidable non-differential misclassification will, however, reduce some of the power given in the table. In any case, the size provides new research opportunities for rare exposures and rare outcomes and will also to some extent allow for studying gene–environment interactions.

ENROLMENT

The National Board of Health did not permit us to contact pregnant women directly in order to recruit

Table I. *Smallest detectable relative risk (RR) in a case-control analysis nested within the cohort, using four controls per case*[a]

Expected cases	Outcome	Exposure prevalence among controls 10% RR	5% RR	1% RR
3,400	All congenital malformations	1.14	1.25	1.60
560	Genital malformations	1.5	1.7	2.7
150	Facial clefts	2.1	2.5	5.0
220	All child cancers	1.8	2.2	4.1
55	Leukaemia	3.1	3.9	9.4

The estimates are based on 80% power to detect the indicated relative risk (or higher) at a testing level of 0.05 in a cohort of 100,000 newborns.
[a]*Sources*: Sundhedsstyrelsen. Sundhedsstatistik 1999: 3 and Basso et al. Am J Epidemiol 1999; 150: 598–604.

them early in pregnancy, and we therefore established collaboration with the GPs, of whom there are approximately 3,500. It was considered acceptable for the GP to give the women an invitation to the study when they came for their first pregnancy visit, usually in weeks 6–12 of pregnancy. Almost all pregnant women have this session with the GP, which is used not only to confirm the pregnancy diagnosis but also to refer women for follow-up by midwife or more specialized care if needed. The GP also carries out certain screening routines including blood sampling. For women who did not receive an invitation at the GP we have a back-up recruitment procedure with the midwives. More than 95% are recruited based on the invitation they receive at their first GP visit.

EXPOSURE RECORDING

We collect information on exposures and minor diseases not registered in medical records by using computerized telephone interviews (Ci_3 and CI_3CATI, Sawtooth Technologies Inc., Evanston, IL, USA). This was done partly to make sure we would interfere as little as possible with the ANC activities, and partly to reduce the number of non-responders and limit non-responses for some of the questions. We knew from previous experience that this was a major problem in cohorts based on self-administered questionnaires. Pilot surveys among pregnant women indicated that they would rather respond to a telephone interview than fill in a questionnaire. An interview also permitted addressing more detailed questions to a subset of the population and left no paper questionnaires to be filed. The telephone interview occurs four times at gestational weeks 12 and 30 and when the child is six and 18 months old. The telephone interviews last on average 18, 10, 16, and 10 minutes.

In addition, at weeks 25–26 of gestation all participants receive a food frequency questionnaire, which was developed specially to address dietary habits among pregnant women in Denmark. It is a 300-item questionnaire that describes intake over the past month. Besides information on food items it includes data on vitamins and food supplements. The questionnaires are processed concurrently by means of scanning.

The content of the interviews and questionnaires was developed in consultation with external experts, including members of our expert committee and steering group. The work was carried out over a four-year period and was subject to several pilot tests. We wanted the interviews to be acceptable, brief and not frightening. We wanted them to cover exposures related to fetal growth as well as to provide detailed data on use of medicine and diseases of the mother and child that were not available from other sources.

Additional exposure information on social conditions and occupational status at the individual level may be obtained from national registers. By using information related to the address, data on air pollution or drinking water quality may also be available.

It will also be possible to get information on redeemed prescription medications obtained by mothers as well as the children by linking our cohort to the National Prescription Database. For this we will need additional permissions, which have not yet been obtained.

LOGISTICS

The project is based on a computerized platform which ensures that all necessary procedures are activated at the proper time, such as sending out letters, activating the telephone interviewers, and keeping track of changed addresses and telephone numbers. This platform is linked to the biobank as well as the questionnaire information. It is written in FoxPro and Access. All personal identifications in the database are based on a personal code different from the personal identification number used in administrative registers. The file with links between the two numbers is kept locked up in a different place.

It was logistically impossible to prepare blood taken at GP surgeries or at delivery in any special way. GPs and personnel at the delivery wards therefore send EDTA-treated whole blood to the biobank using ordinary mail (i.e. without freezing or cooling during transport). Blood samples are thus being transported at outside temperatures for four to 48 hours, but most of the samples reach us within 28 hours. The GP takes blood samples at the routine visits in gestational weeks

6–12 and 24. The umbilical cord sample is taken at the hospital shortly after delivery, usually by the midwife or an assistant nurse. All delivery wards have been asked to keep the blood samples in a refrigerator until they are mailed.

When we receive the blood samples four whole blood spots are put on filter paper. The blood sample is then separated. Plasma and buffy coats are put into liquid nitrogen and in freezers at $-20°C$.

GPs provide a blood sample to our biobank during the first routine ANC visit. As a consequence, we get more blood samples than signed informed consent forms. We were given permission by all the regional Scientific Ethical Committees to send out a reminder to the women if we had not received the signed consent form within two weeks after we received the blood sample. This permission was given only for the last half of the recruitment period.

Participants are recognized as cohort members in the ANC system by a sticker showing our logo, which they receive from us to place on their personal pregnancy record and take to all the contacts they have within the ANC system. We also provide a colourful envelope, distinctly different from the normal type of envelope used for this record. These identifiers are used to signal cohort membership and to make sure that blood samples are taken at their second routine GP visit and after delivery. In addition, they receive a roller that identifies date of expected delivery, interviews, and blood sampling.

For the interviews, we have used CATI based systems, programmed and pilot tested before use. Previous experience from smaller birth cohorts was used in the planning phase, but a number of questions were also included to satisfy research areas for which we had specific funding at the time of planning. The final decision as to the content, sequence, and phrasing of the questions was made by the project coordinators (Jørn Olsen, Thorkild IA Sørensen, Mads Melbye, Sjurdur F Olsen, Anne-Marie Nybo Andersen, and Peter Aaby) together with those in charge of coordinating writing the pregnancy questionnaires (Anne-Marie Nybo Andersen) and the postnatal questionnaires (Jente Andresen).

When planning the study, we established an advisory board with members appointed by the professional organizations involved in the data collection. These were: midwives, GPs, obstetricians, paediatricians, child nurses, and a member from the parenthood organization. A steering committee was established that included some of the funding bodies: the Danish National Research Foundation, the National Board of Health, Statens Serum Institut, and the counties. These members appointed a midwife to take part in the steering committee as well as the project leader (Jørn Olsen).

When women agree to participate in the cohort, they provide a preferred time period for the telephone interview. If they are not reached at this time we try to contact them on three additional occasions, at different hours, within the following two weeks. If we have had no contact by then, we check their addresses and telephone numbers and call them again if the first number indicated was wrong.

END POINTS

Intensive follow-up is done by means of the last two telephone interviews. We ask questions about motor development and diseases as well as questions on breastfeeding and changes in occupational or social conditions in the family.

For the data collection to be affordable, we based most of our outcome data on routine health registers. These cover almost all the population and, in general, are of reasonably good quality, especially for diseases treated in specialized departments. As a new element in the routine birth registration, the National Board of Health included measures of the head and abdominal circumference and the placenta weight at our request in January 1997. From the pregnancy registration within the National Hospital Discharge Registry, we have data on diseases during pregnancy leading to hospitalization, data on birth weight and length, data on gestational age at birth, birth complications, and delivery methods. A more complete description of what is available in the registers is given elsewhere (21–28). We obtain an ongoing update of all hospitalizations for mothers as well as the children born into the cohort by annual linking of the Danish National Birth Cohort with the National Hospital Discharge Registry, which covers inpatients as well as outpatients for all public hospitals (more than 98% of all hospitals). It is also possible to get data on cerebral palsy, infantile autism, childhood cancers, and diabetes by linkage to specialized disease registers designed to cover these outcomes. Since it is easy to locate all cohort members at any point in time we could send out questionnaires or call them in for health examinations if we have the necessary funding. This could be done for all or for a sample, e.g. selected on specific exposure profiles. Such studies have been planned for specific dietary profiles and alcohol consumption. Most of the planned studies will be done as case-control studies within the cohort, which permits better disease classification at this stage of analysis.

We have permission to follow up the cohort for 20 years, and it is possible to extend this permission. It is easy to continue follow-up as long as is desired if permissions are given. Mortality and immigration in the cohort are routinely registered by regular linkage

to the National Population Registry, and unambiguous identification of addresses and phone numbers is possible with linkage to the Civil Registration System.

We expect to have close to complete follow-up of the cohort through these national registers, which are mandatory and cover all.

RECRUITMENT AND COMPLIANCE

Candidates for the cohort are all pregnant women in Denmark who, at their first visit to the GP, want to carry their pregnancy to term and who speak Danish well enough to take part in the telephone interviews. No other exclusion criteria are used.

Table II describes recruitment by December 1999 by counties. The estimated participation rates are given for 1998 and 1999. Except for two counties we are recruiting about 35% of all pregnant women. We expect approximately 10% of all pregnant women not to meet our inclusion criteria. Pilot testing has shown that about 60% of all pregnant women receive the invitation to participate. Those who did not agree to participate stated that the main reasons were lack of time or interest. We expect a response rate of about 60% among those invited (Table II).

Table III presents compliance with the data collection scheme.

We do not expect the cohort to provide a representative sample of pregnant women and the intention is to use the data source for internal comparison. In doing this we rely upon the expectation that forces of selection will not be associated with both the exposure and the outcome since the outcome is not known at recruitment. This assumption will, however, be evaluated.

Table II. *Recruitment and participation rates*

County	Date of permission	Estimated participation rates (%)	
		1998	1999
Copenhagen county	7 May 1997	26	22
Frederiksborg county	6 May 1997	42	37
Roskilde county	22 September 1997	38	32
West Zealand county	7 April 1999	–	19
Storstrøms county	18 February 1997	17	18
Bornholms county	28 January 1997	36	24
Funen county	2 October 1998	–	38
Sønderjyllands county	6 October 1997	16	17
Ribe county	12 May 1998	22	39
Vejle county	29 December 1997	42	42
Ringkøbing county	27 May 1997	32	26
Århus county	29 October 1997	33	36
Viborg county	24 January 1997	42	35
Nordjyllands county	28 February 1997	38	32
Copenhagen city	21 January 1997	27	26
Frederiksberg city	19 February 1997	35	32
All		28	30

Table III. *Compliance with the data collection scheme*

	Time	Percentage
1st blood sample	Gestational weeks 6–12	97
1st interview	Gestational week 12	87
2nd blood sample	Gestational week 24	77
Food frequency questionnaire	Gestational week 25	77
2nd interview	Gestational week 30	88
Umbilical cord blood sample	At delivery	65
3rd interview	6 months after delivery	87
4th interview	18 months after delivery	75

PILOT TESTING

Inasmuch as some members of the health professions were concerned about the telephone interview, we have pilot tested the interviews done during pregnancy. The interviews were in general well accepted and caused no reported additional fear related to pregnancy. By interviewing several GPs and midwives we also have no reason to believe that this interview interferes with the normal ANC routines. Pilot testing has shown that the interviews work well. Nothing indicates that the questions make the ANC more disease oriented than it is. Nothing indicates that the questions lead to concerns that take time from the midwives and GPs.

As part of our quality control of the interview, we included staff members in the interview list at regular intervals without informing the interviewers. In general, our experience with the quality of the interviewing has been good except for a tendency to leave too little time for answering in some situations. This and other aspects of interviewing are taken up at our regular staff meetings with the interviewers and at the training courses for the interviewers that we also conduct.

ETHICS, PERMISSIONS AND COLLABORATION

More than two years were spent on discussing ethics, logistics, funding, and permissions. We received moral support for the project from the Chief Medical Officer, but he wanted the Parliament's ethical council to discuss the project before it was presented to the Minister of Health. This discussion took place on 15 September 1994 and resulted in a supportive statement that also included issues of concern for further debate. A few members of the council started a debate in the public

media before we had even developed a full protocol and had funding in place. This debate presented serious threats to the financial structure of the project not only in terms of the delay it caused but also in terms of the restrictions that followed, and it almost eliminated the option of support from private foundations. It furthermore complicated our negotiations with the midwives and the GPs. Critical arguments were based on a popular belief in the possibilities of predicting future health based on analyses of blood samples, but the debate also included the fear of disclosure of personal information and the fear of making pregnancy "disease" oriented rather than "health" oriented. The debate even raised concern about introducing additional screening into the ANC, although this was never intended.

According to the principles stated by the Ethics Committees, participants have the right to have their data removed from the cohort at any time and the right not to be subject to more telephone interviews or blood sampling. So far less than 25 out of 50,000 have asked to be deleted from the cohort and 8% have been taken out of active follow-up, primarily because they did not carry the pregnancy to term. Children born into the cohort participate on their mothers' consent until they are able to decide for themselves (no later than at 18 years of age).

Our main sponsor (the Danish National Research Foundation) submitted the protocol for peer review by the Medical Research Council and leading international experts in the field in 1995. This review was very positive and facilitated major support from the Danish National Research Foundation, the Danish Pharmaceutical Association and the March of Dimes Birth Defects Foundation.

The local counties (of which there are 14 in Denmark) together with the cities of Copenhagen and Frederiksberg run the public health care system, covering all ANC, deliveries and treatment related to pregnancy. We could not set up a National Birth Cohort without their support and they discussed the project at their council in 1995 and 1996. They recommended that all counties support the study. We sent out a request for support in December 1996, but the decision process was unexpectedly slow in many of these counties. Not until May 1999 did we have support from all counties and a national data collection scheme in operation. We enrolled counties as soon as we received permission (see Table II). So far, the lowest recruitment rates have been seen in Copenhagen and two smaller counties. They also have the lowest compliance with the blood sampling procedures.

CONTACT WITH UNIONS

Almost all GPs and midwives are organized in two respective unions. We had, as is required, the protocol approved by the GPs' Multi-centre Research Evaluation Committee. The union leaders were, on the other hand, not satisfied with the standard fee they received from the counties for taking the blood sample, and they sent a letter to all their members stating this concern and let it be up to each member of the union to make his or her own decision about taking part in the study or not. They did not recommend the GPs to take part in the study (or advise against doing so) but at a regional level some of the local union leaders actually recommended their colleagues not to take part in the study at all. This recommendation was apparently followed by many GPs in at least two counties (Sønderjyllands and Storstrøms counties) and in the Copenhagen area. The midwives' union had a similar ambiguous attitude to the project.

Given these conditions we needed to advocate the project ourselves which was done in numerous meetings, debates, and articles in local and national media. All of this has been time-consuming, costly but also very educational.

As a result of this, pregnant women were not to be recruited by the GP or the midwives, but they would introduce the study by handing out the letter of invitation and the background documents for the study. The women should, after having consulted the father of their unborn child, in their own home and without any external pressure, make up their minds and decide whether they want to sign the informed consent form. The background documents require reading up to four pages of text. The woman also has to mail her signed informed consent form, including her personal identification number (PIN), telephone number and permission for us to use information from her and her unborn child that is available in medical records and registers. This form is mailed in a postage-paid envelope together with a short questionnaire on periconceptional use of medicines, vitamins, and supplements.

PERSPECTIVES

We expect that the need for the cohort data will not disappear: on the contrary these data will be needed much more in the future. We expect no unwanted disclosure of data and we believe the technical options for protecting data to be even better. We think that the arguments for a life course approach to epidemiology and studies on disease causation are so well founded that ANC will have to incorporate this aspect in the future.

We expect that this study will provide new information on the role of infections, diet, genes, and the social environment for serious diseases such as congenital malformations, asthma, childhood cancers, behavioural disorders, and later cancer of the testis and other

diseases in adults. We also expect to learn more about the safety of medicine taken during pregnancy.

EPILOGUE

Our experience has shown that it is possible to include 100,000 participants in this cohort in Denmark. In spite of problems, a large number of GPs, midwives, and pregnant women have taken part in the study. It was a surprise that the start-up was saddled with so many complications in making prevention at the beginning of life more evidence based. On the other hand, once the programme became routine the critical debate stopped. The GPs who decided to take part in the recruitment have remained important participants throughout the long period of recruitment. Also, the midwives became much more engaged in the project over time.

Many of the GPs, midwives, and pregnant women took up the challenge and worked with dedication to make the project a success. We believe that in a few years' time all will realize that this project was a good investment, one whose payback will offer more benefit than most other investments.

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