

Cryptorchidism and Hypospadias in The Netherlands: Are Endocrine Disrupters Involved?

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Thank you Mr Chairmen for your kind introduction, and good afternoon, ladies and gentlemen. I'd like to thank the organisers for inviting me here, and for organising this excellent meeting. It is also a pleasure for me to pay a visit to Japan for the first time.

The subject of my presentation is the prevalence of cryptorchidism and hypospadias, and the role of endocrine disrupters. I realise now that the title for this presentation I gave the organisers is a bit too ambitious. I will not give a definite answer to the question: Are endocrine disrupters involved? But I am working on it!

This presentation will address 2 important aspects in the discussion on cryptorchidism and hypospadias. In the first part of the presentation, I will go over the accuracy of birth defect registries. After showing some of the time-trends and geographical differences reported by birth defect monitoring systems, I will describe the methodological limitations of these data.

I will present to you the results of a unique study in the Netherlands on exact rates of cryptorchidism and hypospadias. These results will be briefly discussed. The second part of my presentation concerns the question: Are endocrine disrupters involved in the aetiology of cryptorchidism and hypospadias? Before we can answer this question, we need to develop a methodology to estimate the very complex exposure in the general population. I will describe the exposure assessment strategy in the ongoing studies in Rotterdam, to illustrate our current approach.

I will end with conclusions and future plans.

Before I continue, I will show you 2 pictures of the 2 birth defects, so that you know what I am talking about:

Cryptorchidism picture

Here you see the genitalia of a young boy. As you can see, one testicle is not in the normal scrotal position, which is the definition of cryptorchidism.

Hypospadias picture

In this slide, you see an example of hypospadias. In hypospadias cases, the urethra does not open on the tip of the glans, but somewhere else on the ventral surface of the penis. This abnormality is usually treated by surgical correction.

Part 1: Accuracy of registries

Okay, let me start part 1 with the question: Are there truly differences in birth rates of cryptorchidism and hypospadias in time, and in place? Several groups in Europe, the US and Asia have reported increasing trends and geographical differences in these abnormalities in the late 20th century. Some examples are given in the following slides.

Examples 1

These figures are taken from a review by Jorma Toppari and co-authors, showing the results of studies in the United Kingdom and Hungary, indicating a doubling of hypospadias rates over a period of 30-50 years. These initial studies were followed by several other reports. Below is a graph of hypospadias rates in a number of Scandinavian countries. I want to point your attention to 3 phenomena that can be seen in these graphs. One is the apparent increasing trend, secondly, you may note the enormous geographical differences in prevalence rates, and thirdly, we can see large variations between successive years within several registries. Some countries seem to produce more reliable rates over the years than other countries.

Examples 2

Here you see another example of rising trends of hypospadias in a large US registry, that used standardised methodology during the whole period.

Examples 3

This slide shows 2 figures from another paper by Paullozi, presenting data of the International Clearinghouse for Birth Defects Monitoring Systems. In the panel on the left-hand side, hypospadias rates for several countries over the period from the late 1960's to 1990's are given. The lowest curve (with asterisks as markers) plots the rates for Japan from 1972 to '96. The curve for Japan is interesting because the rate is relatively low compared with other countries, and because the rates for consecutive years are very consistent, as was also shown in the previous talk by Dr. Hirahara. He showed that the more recent rates were again a little bit higher.

The right panel shows some curves for cryptorchidism, that also show differences in time and place. For cryptorchidism, less data is available, as many registries do not record cryptorchidism. Cryptorchidism rates are very dependent on the age of the boys, as the cryptorchid testicle may spontaneously descent into the scrotum in early life.

We can, unfortunately, not read from these graphs whether the differences in time and location are real, or that they are a result of inaccurate methods.

Problems with interpretation of registry data:

So before we draw conclusions based on registry data, we need to understand the methodological limitations of these data. This slide lists some potential problems that may affect the accuracy of registry systems. I have to emphasise that not all registry systems are equally biased by these problems.

Problems affecting the numerator, which corresponding to the number of cases, are:

- differences in case definition: not all systems use the same criteria for diagnosing someone as a case;
- exclusion of subtypes: some systems exclude minor grades giving lower estimated rates; moreover, the grading of subtypes is not uniform among registries;
- often not all newborns are systematically screened for abnormalities, so that not all cases will be detected, giving an underestimation of the true rate;
- some registries rely on the cooperation of physicians to report cases, which may not give a complete coverage; and
- some registries are based on surgery records. However, not all cases are treated by surgery, and the criteria for surgery may have changed.

Some problems may affect the denominator, which represents the size of the reference population:

- as not all newborns are screened, the denominator has to be approximated, for example based on the estimated size of the population that uses a certain hospital.
- another problem is that not all cases will be reported within this reference area; for instance, some mothers may have given birth to their son outside the defined reference area, or may chose to deliver in another hospital.

What is the magnitude of these problems?

It is important to recognise and understand the magnitude of these problems in registries. We have to realise that most published data are based on such registry systems.

The magnitude of these problems may be analysed by comparing registries with complete systematic screening of birth cohorts.

The Rotterdam cryptorchidism and hypospadias study

We performed such a comparison in the city of Rotterdam. The first aim of the study is relevant for part 1 of my presentation. The other 2 objectives, concerning the role of endocrine disrupters, will be covered in the second part of the presentation. This first aim of the study was to accurately assess the prevalence of hypospadias and cryptorchidism by examination of a complete birth cohort.

Design of the Rotterdam study

The dsIn a cross-sectional design, we studied the occurrence of hypospadias and cryptorchidism in more than 7,000 consecutive male births in Rotterdam in 2 years time. The 7,000 boys were subjected to a standardised examination at Child Health Care centres around the age of 1 month. These Child Health Care centres carry out the national preventive child health programme, which includes for example vaccinations against infectious diseases. These services are free of charge, and 99% of all newborns participate. All cases were referred to a paediatric urologist in our childrens' hospital for verification and grading of the abnormality.

To study the role of endocrine disrupters, which will be covered in the second part of my presentation, the cases identified in the birth cohort are included in a nested case-control study, that will compare approximately 150 cases with 450 controls.

Cases and selected controls are visited at home by a research nurse. During this home visit, data is collected on exposure to endocrine disrupters by an interview and collection of blood of the newborn and this mother.

Methods: standardised examination

Hypospadias were graded by experienced paediatric urologists, as shown on the left. The glandular grades are usually considered as minor, and the others as major. It has been suggested that the increasing trend in hypospadias rates in some registries were the result of increased reporting of cases of minor severity. This hypothesis could not be tested properly, because the frequency distribution of subtypes was unknown in most cases. Therefore, we decided to include all these subtypes in our study.

Also the location of the testicle was examined in a standardised manner, which is shown here on the right.

Results: occurrence of cryptorchidism

This table shows the results of the cryptorchidism examination in 7,292 boys. The presence or absence of cryptorchidism was reported for 95% of the boys that visited the child health care centres in

Rotterdam. In 79 of the boys, uni- or bilateral cryptorchidism was confirmed by the paediatrician, which gives a rate of 1.1%

Results: occurrence of hypospadias

This table shows the occurrence of hypospadias subtypes in the same number of boys, after verification by the paediatric urologists. Hypospadias was present in 53 boys, giving a rate among boys of approximately 0.7%. Cases suspected by child health care physicians were confirmed by the paediatric team in 88% (that was 53 out of 60).

The ratio of minor to major cases was 12 versus 36, indicating that 25% of the cases was of minor severity. In 80% of boys born with hypospadias, surgery was required because of problems with urination, or because of bending of the penis. Also in 25% of the glandular cases, surgical correction was indicated.

In the following few slides, I will discuss our results in comparison with other reports in the scientific literature.

Discussion 1

The cryptorchidism rate in Rotterdam of 1.1% is in correspondence with the international literature.

72% of the cases suspected by child health care physicians were confirmed by the expert paediatrician. The child health care centres appear to be a good institution for systematic screening for cryptorchidism and hypospadias in the Netherlands. The difference with the paediatricians' judgement is most probably due to the time lag between both examinations, as many cryptorchid testicles are known to descent spontaneously during early life.

Discussion 2

The most significant finding in our study is the high rate of hypospadias. We found a rate of 0.7%, which is much higher than what was expected on the basis of Eurocat, which is the European Register of Congenital Abnormalities, that collects data from 16 European regions, including the Netherlands. The European registry excludes cases of glandular hypospadias. If we exclude glandular cases, the rate is still 4 times higher than the Eurocat rate for the Netherlands.

Our study implies that the European birth defect registry has substantial underreporting. We have to realise the reports on trends in hypospadias are all based on such registry data, that are sensitive to changes in case ascertainment.

It has been suggested that the 2-fold increase in hypospadias in the United States over the past 50 years may be due to a trend to report minor cases more effectively in recent years. This hypothesis could not be tested, as the subtypes of hypospadias were unknown for the majority of cases. In our population, the ratio of minor to major was only 1/3. If this ratio would also apply to the US systems, the doubling of hypospadias prevalence in these systems could not be caused by a higher proportion of minor cases.

Discussion 3

In the same period that we did our study, a comparable study on systematic screening for hypospadias was conducted in Finland. In this study, only 19 cases were found in a cohort of nearly 6000 boys. We found a 2 fold higher rate in our study, which will be published in the journal Human Reproduction early next year.

The 2.2 fold geographical difference based on the first 2 accurate hypospadias prevalence rates warrants further study on the explanation of this finding. It would be very interesting to study the births

rates in other countries in a systematic manner, to see whether for instance the rate in Japan is truly lower than in other parts of the world, and if we can identify differences in environmental and genetic risk factors between countries.

Presentation structure

As I said in the beginning, the second part of my presentations deals with the difficult task of exposure assessment in a population based study. I will summarise the issues that are, in my opinion, important in that respect. I will describe the exposure assessment strategy in the ongoing studies in Rotterdam, to illustrate our current approach.

Evidence for the role of ED in cryptorchidism and hypospadias

Is there evidence for a causal relationship between endocrine disrupter exposure and the occurrence of urogenital abnormalities? Well, in wildlife and animal experiments these effects have been shown. In the human, however, there is only one such example, and that is the occurrence of cryptorchidism and hypospadias in sons of mothers that were treated with pharmacological doses of the very potent synthetic oestrogen DES (di-ethyl-stilbestrol).

For the general population however, the evidence is only circumstantial. Epidemiological research has shown an increased hypospadias risk in certain subgroups, that may be associated with exposure to endocrine disrupters. For example, hypospadias was more frequent in sons of mothers that had a vegetarian diet during pregnancy, for which elevated exposure to natural phyto-oestrogens has been suggested as the cause. Also, an increased hypospadias risk was observed in children born after assisted reproduction technologies such as IVF. In this case, foetal exposure to the hormones given to the mother for ovulation induction has been named as possible explanation.

So far, only epidemiological associations have been described in population studies. No link between exposure and these health endpoints has been demonstrated. A first step to move from association to causation is to prove the causal role of endocrine disrupters. In other words, we have to assess the actual exposure. And this is a complex task.

The exposure assessment issue

The high doses and well-defined exposures to single chemicals in animal experiments are not representative for the general human population. The human is typically exposed to complex mixtures of substances at low concentrations, which also varies during life.

It is a challenge to develop an integrated model for assessment of human exposure.

Aspects of integral exposure assessment

All of us here are exposed to endocrine disrupters from several sources, such as nutrition, environment, occupation and consumer products. Unlike the average animal experiment, the human is simultaneously exposed via different routes, such as the dermal and inhalation pathways. As it is not feasible to measure all exposures through these pathways and sources on the individual level in community-based studies, we have to use other methods.

As an alternative method, we can model the exposure of subjects by using questionnaires on nutrition, occupation etcetera. We can link the questionnaire data with exposure levels by using existing data. For example, databases on occupational exposure levels to chemicals can be used. If insufficient toxicological data is available, specific environmental monitoring can be performed. Moreover, we can

run bio-assays on serum samples, to see whether the estimated exposure correlates with the internal dose of endocrine disrupters.

Now I will illustrate this approach by presenting the current exposure assessment strategy in our ongoing cryptorchidism and hypospadias study.

Exposure assessment in the Rotterdam study

This slide shows the different aspects of endocrine disrupter exposure and other risk factors that are included in our questionnaire, that is used for interviewing parents of cases and control boys. It includes personal characteristics (education, address); a nutrition section on phyto-oestrogens (for example soy-products), a detailed occupational history, medical factors, etc.

Questionnaire: nutrition section

The nutrition section was specifically designed for our study. Food products were selected in the following steps: (1) the product is known to contain isoflavonoids or lignans, which are the most potent phyto-oestrogens, (2) the product is used with a relevant frequency in the local population, and (3) the concentration of isoflavonoids or lignans is available from previous research or databases. The intake can then be calculated by multiplying frequency of use, portion size and concentration.

Questionnaire: occupational and environmental section

Also for the occupation section, we can use existing knowledge on jobs with relevant exposure. Expert opinions of occupational hygienists provide us with estimated levels of exposure. This is called a job-exposure-matrix. And for certain jobs, quantitative exposure data is available in databases.

Also for estimation of environmental exposure, data are available from monitoring systems on pollution of outdoor air and drinking water.

The Rotterdam study: biomonitoring

In addition to the questionnaire, we have also collected blood of boys and their mothers. We have developed an oestrogen receptor mediated reporter gene assay to measure the total internal dose of oestrogen-like bioactivity in serum, which measures both exogenous and endogenous activity. The level of endogenous activity is analysed with the regular oestradiol assay that is also used in clinical practice. We can then calculate the exogenous oestrogenic activity by subtracting the endogenous fraction from the total activity measured by the bioassay.

The assay has been optimised for small volumes, because the amount of available blood in epidemiological studies is usually limited. Only 100 micro-liters of serum is required.

Early next year we will start using the assay for analysis of blood from cases of cryptorchidism and hypospadias, the controls, and their mothers. We can use the questionnaire data to link the internal dose with possible sources.

The biomonitoring will be validated against the questionnaire and vice versa.

General conclusions

This brings me to the summary of my presentation. In conclusion, I would like to say:

- (1) for accurate monitoring of cryptorchidism and hypospadias rates and trends, complete case ascertainment including classification of severity is warranted. Until very recently, only registry data were available for this purpose. Based on registry data only, it is hard to prove a

widespread increase in cryptorchidism and hypospadias. It would be interesting to compare rates of systematic screening studies between countries.

- (2) Secondly, the causes for the 2 abnormalities, and for the differences in time and place need to be explored.
- (3) My third last conclusion is that to be able to link health effects to endocrine disruption, we need to develop appropriate exposure assessment strategies and markers.

Future plans

- My future plans are given in this slide. Early next year we will start with the analysis of risk factors in our case-control study. So next year around this time, I hope to be able to give you a more definite answer to the question: Are endocrine disrupters involved.
 - Last week, a large cohort study on 8,000 pregnant women and their offspring has started. This study has an anticipated follow-up until children reach adulthood. In this study, we can do etiological studies on the effects of exposure to endocrine disrupters on reproductive health, but also on neurological development, growth, etcetera.
 - A third plan is to further develop the integral exposure assessment strategy, including further validation of the oestrogen assay.
- We would also like to study the geographical differences in hypospadias rates in more detail.

For all of these plans, we are looking for co-operation with other groups that share our interests.

Research partners

This last slide shows my research partners in the ongoing studies in Rotterdam.

I'll finish here. Thank you for your attention.

Q&A

Lancaster: Thank you very much, Dr. Pierik. It is good to say that a group is setting out to actually test some specific hypotheses based on exposure information and not just the outcome. Do we have any other questions, please?

Daston: Interesting talk, Frank. I had two questions. One is: in your case-control study that you are planning, are the controls going to be normal controls or are they going to have an unrelated malformation?

Pierik: They will be normal boys that do not have the abnormalities. That will be the criteria and they will be matched for age.

Daston: What are you going to do in terms of controlling for recall bias in that case?

Pierik: I think that the only way that we could do it is that we included the cases after birth. The ideal situation is that we would select the cases — not select, but we just follow is what we are going to do in the coming study to prevent these problems of bias. In the current study, that is something that might play a role. We invite parents to participate in both in cases and controls, and for controls there may be other information known by the parents.

Daston: It would be interesting to see whether — I do not know whether this is possible in your study — but to use two control groups: a normal control and a malformed control with an unrelated malformation.

One of the real raging controversies that I see in the teratology epidemiology literature is how to control for recall bias and I think it is especially difficult in this case because some of the hypospadias may be fairly minor, and as you said, surgically correctable. The recall bias might not be as severe as in some other cases, so it might be interesting to inform both your question and the question of the importance of recall bias.

The second question that I had for you was: the previous speaker had shown strong association between hypospadias and low birth weight and/or prematurity. Do you see the same thing in your data set?

Pierik: We are waiting with analysis of risk factors until we have our groups of cases and controls complete. We hope to finish that beginning of April. Then, we should have the 150 cases, and then we will look into these things because it is very well known that probably the most prominent risk factor is low birth weight. There are also effects of ethnicity and several other things. For example, there has been a higher risk found in cases after assisted reproduction. So that is also something we will look into.

Lancaster: I agree with your first question. I think you made a very important point about the need for a malformed control as well as a normal control because endocrine disrupters are now being fairly widely publicized, so it might not be too much to expect that in a well-informed Dutch population the women may well be aware of the hypothesis. So I think it is very important.

Pierik: Yes, it is important, but it is difficult because the questions are sort of a general nutrition questionnaire, and they do not know in what food product would be the most phytoestrogens. But for jobs you can see that people really try to think of things they might be exposed to. It is something that might play a role.

Lancaster: One final comment from me: you said that the rate in Rotterdam of hypospadias was about four times the EUROCAT average, but in fact, I know in the International Clearinghouse, although I know that hypospadias is almost exclusively a male sex specific abnormality, we actually report the rates per 10,000 total births. I just wonder in comparing your figures, in which

your denominators were boys, but in the EUROCAT data...

Pierik: If we can switch the projector on again, I have an excellent slide on that subject that shows those rates, also. If you would calculate it, here you see the rates for 16, I think, different European regions, which also shows a lot of variation. You see that the average is 1:1,000, which usually we produce it in 10,000, so it would be 10:10,000, whereas the rate in the Netherlands was 38:10,000 in Rotterdam.

That is including females. For us it is the same: we have included females; it is 38:10,000.

Lancaster: Good, thank you very much, Dr. Pierik for your presentation.