**Project proposal:**

Fever during pregnancy and risk of congenital malformation. A cohort study.

1 **Aims**

The aim of this project is to study the relation between fever during pregnancy and the risk of congenital malformations. Specifically, the association between period, duration and intensity of fever and the risk of specific malformations will be examined.

2 **Background**

Each year more than 8 million infants worldwide are born with a serious congenital malformation (CM)(1), approximately 40% of which are genetic or partly-genetic in origin and approximately 10% of which are due to known teratogens. However, approximately 50% of CMs are of unknown origin (2).

Congenital malformation is the leading cause of neonatal death in the USA, Israel, Canada and the UK (3, 4) and generates significant hospitalization (5). With the health transition (decreasing under 5 mortality rates in low and middle income countries) CMs are adding substantially to infant mortality in middle income countries as well (3). In order to achieve the United Nations Millenium Development Goal 4 - Reduce Child Mortality (6), it has been concluded that it is necessary for the international community to do more to reduce neonatal deaths (7).

In order to prevent CMs it is necessary to know the causes. The results of experimental animal studies indicate that maternal hyperthermia is potent teratogen. Guinea pigs and rats have increased risk of birth defects after exposure to hot environments, resulting in elevated body temperature (8-11). The range of defects described in animal experimental studies, using a variety of species are similar to those reported in human epidemiologic studies (12). Several retrospective studies have observed association between fever/common cold/hyperthermia and various congenital malformations. Associations concerning neural tube defects (NTDs) (e.g. anencephaly, spina bifida,
meningo/encephalocele) are the most frequently and consistently reported (13-23). Also frequently reported are cleft lip-palate (13, 17, 20, 24-28) and cardiovascular defects (e.g. atrioseptal defect, ventriculoseptal defects, transposition of the great arteries, left heart hypoplasia) (20, 29, 30). Many other CMs are reported more sporadically, including Hirschsprung disease (31), renal defects (agenesis, obstructive defects, duplication defects) (32), isolated congenital cataract (33), microftalmia, microcephaly, micrognathia, and midface hypoplasia (24) and anorectal malformations (34). However, most studies have not addressed the issue of timing, duration or intensity of fever and have used very broad groups, e.g. self reported fever or “cold” from one month before to two months after conception. In addition some are prone to recall-bias due to delay to data collection (2-12 years) (35, 36).

Conclusions from the fewer prospective studies have been less uniform, as some report no association between fever and any CM (37, 38), but others indicate an association with NTDs (16, 18, 39) and overall increase in “serious or potentially serious” birth defects (including NTDs, pyloric stenosis, congenital heart disease and more) (13). Most of the prospective studies lack information on temperature and timing, and only some control for confounders.

A systematic review and meta-analysis from 2005 concerning both retrospective and prospective studies of hyperthermia and NTDs reported an overall OR of 1.92 (95% confidence interval = 1.61-2.29) for a fever episode in early pregnancy (40).

Moreover, fever during pregnancy is common. Nybo Andersen et al. reported that 18,5% of women experience fever during the first 16 weeks of pregnancy (41). Fever was, however, not associated with miscarriage in this prospective study.

Recent case-control studies have shown both protective (20, 26, 28, 33, 42) and deleterious (21-23, 27, 32, 36) effects on the risk of specified CMs when fever has been treated with antipyretics. Heterogenicity exists concerning type of antipyretic, timing and dose of exposure. Certain antipyretics have been associated with adverse pregnancy outcomes (43). We feel that it is important to
ascertain to what extent fever is a teratogen before clinical advice is given to pregnant women advocating antipyretics, as some researchers suggest (20, 26, 33).

Animal studies indicate that the minimum teratogenic dose of heat is an increase in core temperature of 1.5 °C. Also, they indicate that there is a dose-response relation between temperature and duration of hyperthermia. However, the heat threshold required to generate various CMs in different species varies significantly (44). This variability precludes extrapolation between species and in particular to humans (45).

2.1 Possible mechanisms of action
A number of pathogenic mechanisms exist leading to heat-induced developmental defects. These include cell death, particularly of proliferating cells, e.g. the rapidly forming head-fold and anterior neuropore regions. This has been demonstrated to lead to NTDs and microcephaly in rats and guinea pigs (44, 46). Other mechanisms are disruption of cell-membranes that has been reported to result in encephaloceles of the brain, disturbances of neuronal crest-cell migration, believed to result in various malformations, including the heart and face. Also cell division cycle delay, leakage and oedema following vascular disruption and placental infarcts have been proposed as causes (45).

Cells exposed to hyperthermia react with the heat shock response, producing heat shock proteins (chaperones) that protects the cell from aggregating denatured proteins (46). Research indicates that aspirin pre-treatment of mice exposed to hyperthermia increases the teratogenic effect, possibly due to inhibition of prostaglandins used in initiation of the heat shock response (47).

3 Design
A prospective cohort based on the Danish National Birth Cohort (DNBC) study, a research database of 100,418 pregnancies included from 1995 – 2002. Women were followed from the first trimester through pregnancy with follow-up of the children. Information was collected using computer assisted telephone interviews twice during pregnancy. All interviews comprised detailed information on fever concerning timing pertaining to gestational week, highest
measured temperature, duration of fever and number of febrile episodes. Women were also asked if they had taken any kind of medication during pregnancy, what it was and when.

Recorded pregnancy outcomes include more than 2000 children with congenital malformations. Information concerning these stem from two sources: Women who indicated that their offspring suffered from any kind of CM received a detailed questionnaire about CMs. These questionnaires were evaluated case-by-case by a pediatrician. Furthermore, diagnostic Q-codes concerning congenital malformations (DQ00-DQ89) from the Danish Hospital Discharge Registry were traced on all live-born children in the cohort, thus giving us the opportunity to also evaluate congenital malformations that are often not diagnosed at birth, e.g. certain types of cardiovascular malformations.

The study will be conducted using data from mothers of live-born singletons. Women lacking information on fever and women using known teratogenic medication and children with known pre-conceptional chromosomal abnormalities will be excluded.

Possible associations between overall and specific congenital malformations (e.g. anencephalus, spina bifida, cleft lip/palate) and timing, intensity (temperature), duration and number of febrile episodes will be examined using logistic regression. Also we will examine whether the use of antipyretics alters the associations. If an association is found, we will try to exclude mothers known to have suffered from an infection with a teratogenous agent (i.e. toxoplasmosis, rubella, cytomegalovirus, herpes) to assess whether this could be the cause.

A priori we have decided to control for the following confounders; mothers age at conception, in vitro fertilization (48), diabetes mellitus (49), use of alcohol, smoking, socioeconomic status, reumathoid arthritis (50), and effect modificators; child sex, epilepsy (51, 52), use of folates.

To our knowledge, no other population-based, prospective cohort study exists assessing the association between fever in early pregnancy and risk of congenital malformations, with the information required for confounder-control, and evaluation concerning temperature and specific timing of exposure. Thus, this
study has the potential to add significant knowledge to the field of fever/hyperthermia-induced CM.

4 Publication
The results will be published as a paper in a peer-reviewed international journal within the field of obstetrics, pediatrics or epidemiology.

5 Ethics and approvals
The Scientific Ethical Committee approved the data collection for the Danish National Birth Cohort. Approval to use the data for this specific study will be obtained from the DNBC before initiation and so will approval from The Danish Data Protection Agency (Datatilsynet).

6 Time schedule
Spring 2011: Fundraising, approval from the Danish Data Protection Agency. Approval from the DNBC steering committee. Literature review.


7 Researchers and organisation
Anne-Marie Nybo Andersen, MD, Professor, PhD. and Jesper Kjaergaard, MD.

Work facilities (computer, desk) will be provided by Section of Social Medicine, Department of Public Health, University of Copenhagen.
8 Reference List